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Acute Toxicity of Smoke Screen Materials to Aquatic Organisms, White Phosphorus-Felt, Red Phosphorus-Butyl Rubber and SGF No. 2 Fog Oil

Final Report

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AQUATIC ORGANISMS, WHITE PHOSPHORUS-FELT,
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FINAL REPORT

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The acute toxicity of three obscurants was determined for nine freshwater organisms. The materials tested were white phosphorus-felt smoke, red phosphorus-butyl rubber (RP-BR) smoke, and smoke generator fuel (SGF) No. 2 fog oil (bulk and vaporized). The chemistry of WP-F and RP-BR smoke in water and the resulting effects on aquatic organisms are similar. Combustion of these two obscurants and their		

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deposition in water leads to the formation of many complex oxy-phosphoric acids. Rates of hydrolysis of these complex products to ortho-phosphate were inconsistent and unpredictable over time. These products acidify water and produce toxic effects after exhausting the buffering capacity of the water. The 96-hr median lethal concentration (LC50) values for fish ranged from 3.9 to 5.1 pH units. The values for invertebrates ranged from 3.4 to 5.5. Algal growth was inhibited at pH levels less than 6.0.

Acute 96 hr tests using Daphnia magna with neutralized and non-neutralized exposure solutions indicated that the presence of unidentified toxic component(s) acted independently of pH. Non-pH related toxicity was observed in solutions containing an excess of 50 mg/L total P.

At pH levels of 6.0 to 7.0, phosphorus combustion products precipitated out of solution leading to a bimodal toxic response in extended 96-hr tests with Daphnia magna. These effects appeared to be caused by accumulation of the precipitated material in the gastrointestinal tract of exposed organisms. The effect was not observed in exposure solutions that had aged for 26 to 28 days. The bimodal response may be unique to small filter-feeding organisms.

Additions of phosphorus into aquatic systems can lead to stimulation of algal growth as long as the resulting pH is not toxic. Stimulation of algal growth to nuisance levels is a potentially serious adverse effect resulting from the deposition of phosphorus smoke into aquatic systems.

Most components of fog oil had low solubility in water. Saturation was apparent at approximately 0.1 to 0.3 mg/L total oil. The composition of the organic constituents of oil-water mixes was different than what would have been predicted from the composition of the bulk fog oil. These differences reflect the different solubility of the lower molecular weight constituents relative to the total composition of the oil. Vaporization had no demonstrable effect on the chemistry or toxicity of the fog oil.

Neither the bulk fog oil nor the vaporized fog oil was acutely toxic to freshwater animals at concentrations less than 10 mg/L total oil. In oil-water mixes in excess of 1.0 mg/L total oil, fog oil quickly separated and floated to the surface. The primary hazard associated with vaporized and bulk fog oil was the physical effect of oil fouling the organisms. Impairment of swimming in first instar D. magna was observed at nominal concentrations of 0.03 to 0.10 mg/L total oil.

Concentrations of bulk fog oil in excess of 2.4 mg/L total oil significantly inhibited algal growth in two of the three batches tested. Vaporized fog oil at concentrations as high as 2.38 mg/L total oil had no apparent effect on algal growth. Because the concentrations at which these effects were observed exceeded the solubility of fog oil in water, it is not possible to attribute the inhibition to chemical toxicity or to a physical effect of the oil.

Photolysis increased the concentration of water-soluble components of the fog oil. Photolytic products formed most likely include aromatic ketones and carboxylic acids. Acute toxicity was demonstrated in oil-water mixes (~10 mg/L total oil) of photolyzed bulk and vaporized fog oil. No difference in toxicity was observed between photolyzed and non-photolyzed dilutions of OWM at comparable levels of total oil.

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EXECUTIVE SUMMARY

The acute toxicity of three obscurants was determined for nine fresh-water organisms. The materials tested were white phosphorus-felt smoke, red phosphorus-butyl rubber (RP-BR) smoke, and smoke generator fuel (SGF) No. 2 fog oil (bulk and vaporized).

The chemistry of WP-F and RP-BR smoke in water and the resulting effects on aquatic organisms are similar. Combustion of these two obscurants and their deposition in water leads to the formation of many complex oxy-phosphoric acids. Rates of hydrolysis of these complex products to ortho-phosphate were inconsistent and unpredictable over time. These products acidify water and produce toxic effects after exhausting the buffering capacity of the water. The 96-hr median lethal concentration (LC50) values for fish ranged from 3.9 to 5.1 pH units. The values for invertebrates ranged from 3.4 to 5.5. Algal growth was inhibited at pH levels less than 6.0.

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Additions of phosphorus into aquatic systems can lead to stimulation of algal growth as long as the resulting pH is not toxic. Stimulation of algal growth to nuisance levels is a potentially serious adverse effect resulting from the deposition of phosphorus smoke into aquatic systems.

Most components of fog oil had low solubility in water. Saturation was apparent at approximately 0.1 to 0.3 mg/L total oil. The composition of the organic constituents of oil-water mixes was different than what would have been predicted from the composition of the bulk fog oil. These differences reflect the different solubility of the lower molecular weight constituents relative to the total composition of the oil. Vaporization had no demonstrable effect on the chemistry or toxicity of the fog oil.

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The three obscurants tested have the potential for adverse environmental effects. On a relative scale, however, none of the materials can be considered highly toxic until very large quantities (e.g., >10 mg/L) enter freshwater systems. A more extensive assessment requires evaluation of the amount of material released to the environment, the site-specific characteristics of the receiving waters, and the biota inhabiting the site.

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1.0 INTRODUCTION

The U.S. Army uses several different materials to generate smoke screens during military maneuvers. Three of the materials currently used are red phosphorus-butyl rubber (RP-BR), white phosphorus-felt (WP-F) and SGF (smoke generator fuel) No. 2 Fog Oil. The phosphorus-based obscurants are used in incendiary devices that are deployed in shells (WP-F) or in canisters (RP-BR). Fog oil smoke screens are produced with portable generators, such as the M3A3.

Use of obscurants during routine training operations may result in the contamination of bodies of water. To assess potential environmental hazards, researchers at Pacific Northwest Laboratory evaluated the acute toxicity of RP-BR, WP-F, and fog oil obscurants to nine different aquatic organisms. The principal vector of exposure involved the generation of smoke screens and the subsequent deposition of their constituents into a body of water. Spillage of fog oil directly into a body of water also constituted a direct vector of exposure. Indirect routes of exposure included the deposition of smoke screen constituents onto vegetation and soil followed by precipitation and runoff into a body of water.

The research project was divided into two phases. In Phase I, suitable dosing methods were developed for introducing the materials into water. In addition, appropriate chemical analyses were developed for monitoring the materials in water. Several toxicity tests with *Daphnia magna* were conducted to investigate the influence of aging and the buffering capacity of water on the toxicity of the phosphorus-based materials. The effects of photolysis on the chemistry and toxicity of bulk and vaporized fog oils were also evaluated.

In Phase II, a battery of screening tests and definitive toxicity tests were performed on nine different species of freshwater aquatic organisms.

2.0 METHODS

2.1 TEST MATERIALS

Three different materials were tested in this project: red phosphorus-butyl rubber (RP-BR), white phosphorus-felt (WP-F), and SGF (smoke generator fuel) No. 2 fog oil.

The RP-BR pellets and the WP-F wedges were obtained from Oak Ridge National Laboratory. The RP-BR pellets were 95% RP and 5% BR. The pellets had a 1.25% coating of an insulating oil and 1% talc for optimum field performance. The WP-F wedges were originally removed from a munition supplied to Oak Ridge National Laboratory for a previous study. The RP-BR was stored dry and the WP-F wedges were stored in distilled water.

The U.S. Army supplied PNL with three drums (batches) of SGF No. 2 fog oil (Table 1). Five one-gallon amounts of each batch of fog oil were

TABLE 1. PROJECT IDENTIFICATION OF THREE BATCHES OF
SGF NO. 2 FOG OIL

Batch	Barrel Designation	Amount
Batch 1	F62 Fog Oil M11 F-12070A & AM1 Type SGF-2 9150-00-261-7895 Mar 80 Batch 984 DLA 600-80-C 0829	~39 gal. (open drum, oil was brown)
Batch 2	Fog Oil M11 F-12070A & AM1 Type SGF-2 9150-00-261-7895 Sep 82 Lot F-2-9-18 DLA 600-82-C-1478	~33 gal. (open drum, oil was yellow)
Batch 3	F-62 Fog Oil MIL-F 12070B Type SGF-2 9150-00-261-7895 Lot #1 DLA Goo-83-C-1284 Date MFD 7-83	55 gal. (full unopened drum, oil was transparent like mineral oil)

transferred to a clean amber bottle for storage. The air space in the bottles was purged with N_2 , and then the bottles were refrigerated.

The N_2 was replaced each time fog oil was removed from the bottles. The drums were also purged of air with N_2 and refrigerated.

2.2 AEROSOL GENERATION

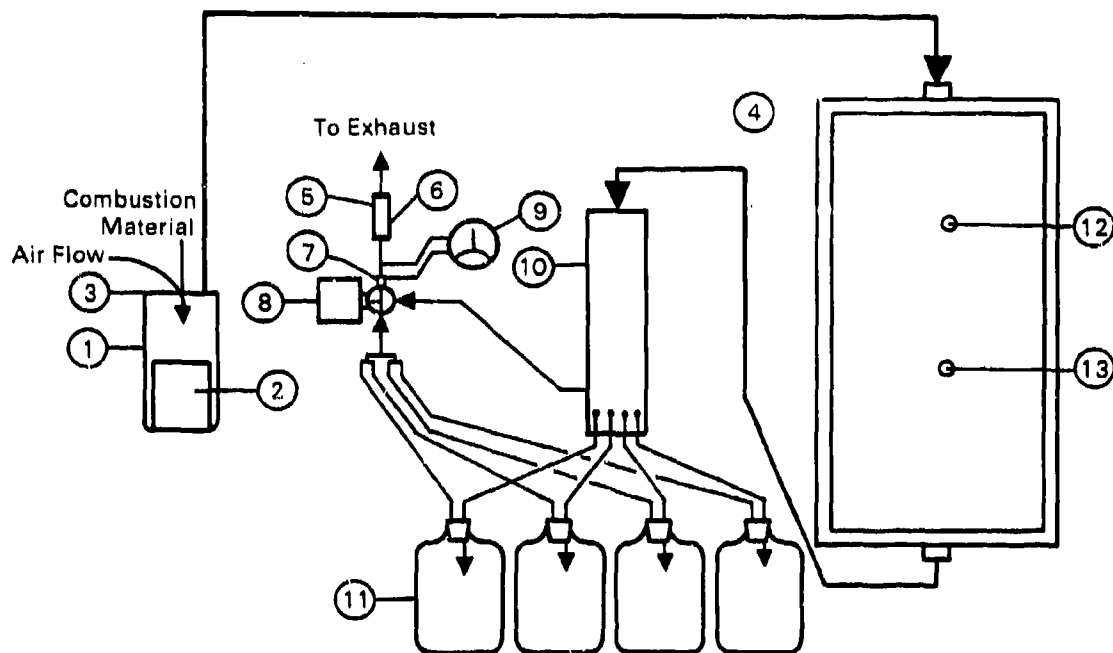
This section describes the methods used to generate aerosols for the preparation of exposure solutions. Aerosol generations (vaporizations) of fog oil are identified as FO-1 through FO-4. Combustions of WP-F and RP-BR are identified as WP-1 through WP-16 and RP-1 through RP-16. The combustions are routinely referred to as "burns."

2.2.1 Red Phosphorus-Butyl Rubber and White Phosphorus Felt

The same system was used to generate smoke from white phosphorus-felt and red phosphorus-butyl rubber (Figure 1). Pellets of RP-BR and cubes (~1 cm³) of WP-R were ignited by passing them through a propane flame and then dropping them onto a bed of sand in a bell jar where they burned to completion. Since these burns were typically of short duration, this task was performed manually. The smoke was drawn from the bell jar through a 36-ft³ buffer chamber by a vacuum pump at a flow rate of approximately 100 L/min. Chamber airflow was monitored by measuring the pressure drop across an orifice in the chamber exhaust line. The buffer chamber smoothed out fluctuations in the monitored smoke concentration caused by the intermittent addition of pellets. Smoke from the chamber was directed to either four 54-L carboys containing the test water or to exhaust, depending on the position of the system's three-way ball valve. Flow rates through the four carboys were about 25 L/min each for a total of 100 L/min. Flow rates were calibrated using a Singer dry test meter.

The concentration of phosphorus smoke in the buffer chamber was determined with a GCA RAM-1 real-time aerosol monitor connected to an in-line TSI model 3302 100:1 diluter. Flow through the RAM/diluter combination was maintained at 5.0 L/min using the RAM's internal flowmeter. The aerosol monitor was calibrated to provide data on the phosphorus smoke concentration by comparing the monitor's output obtained on an analog strip chart recorder to that obtained gravimetrically using filter pad grab samples (Figure 2). Gelman type AE 25-mm glass fiber filters were desiccated, weighed, and inserted into an open-faced filter holder positioned centrally inside the exposure chamber. Several 1- to 2-min grab samples were drawn at 1.0 L/min through the filters. The filters were then redesiccated and reweighed. Calibration data for the aerosol monitor were calculated by dividing the net aerosol mass on the filter by the calibrated volume of air pulled through the filter.

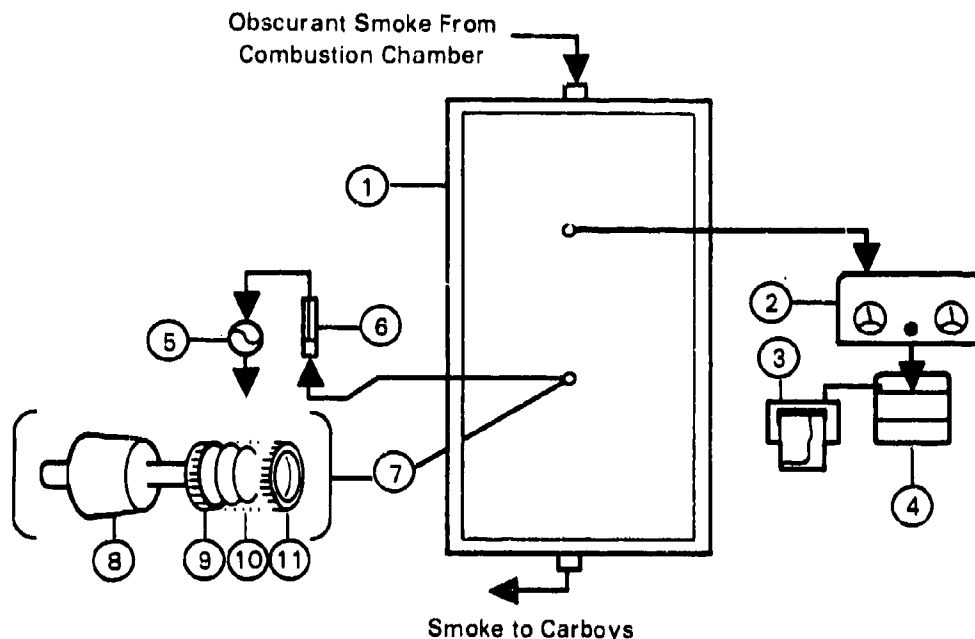
In a typical phosphorus burn, WP-F cubes or RP-BR pellets were ignited and placed in the smoke generator until the smoke concentration in the buffer chamber reached maximum levels as recorded by the aerosol monitor.



- | | |
|--|---------------------------------|
| 1. Generator Bell Jar | 8. 3-Way Valve |
| 2. Sand Filled S/S Beaker | 9. Magnehelic Vacuum Gauge |
| 3. Bell Jar Lid | 10. Manifold (3.5 Liter Volume) |
| 4. Standard Battelle/Hazleton 1.2 m ³ | 11. Carboys |
| Animal Exposure Chamber | 12. Monitor Port |
| 5. Air Actuated Vacuum Pump | 13. Calibration Port |
| 6. Regulated Air Input | |
| 7. Airflow Restriction | |

Figure 1. Phosphorus Smoke Generation System.

The smoke was then diverted to the four 54-L carboys containing the test water, where it settled into the water and onto the sides of the carboys. When the exposure was completed (typically after 10 or 15 min of burn time), the four 54-L carboys were by-passed. The burn was terminated and the four 54-L carboys were removed from the smoke generator system, capped, and placed on their sides for 1.5 hr to allow the smoke to settle into the water and onto surfaces of the carboys. After 1.5 hr, the sides of the carboys were rinsed by rolling and inverting the carboys. Gravimetric grab sample analysis confirmed that no smoke remained in the head space above the water after 1.5 hr.



1. Animal Exposure Chamber
2. 100:1 Aerosol Diluter
3. Chart Recorder
4. GCA Model RAM-1 Aerosol Monitor
5. Vacuum Pump
6. Rotameter
7. Sample Probe
8. Stopper
9. Filter Holder Base
10. Filter Element
11. Filter Holder Ring

Figure 2. Filter Sampling Device for Phosphorus Smoke Generation System.

Studies with fish or Hexagenia required larger volumes of water than could be had been exposed in 54-L carboys. This necessitated larger volumes of phosphorus smoke and, hence, a larger smoke exposure chamber than could be provided by the four 54-L carboys. A special 760-L (200-gal) Fusion Rubbermaid® carboy (Pt. No. 1185, Statesville, North Carolina) was substituted for the 36-ft³ buffer chamber shown in Figure 1. This chamber served as a buffer chamber, sampling chamber, and smoke exposure chamber

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for the test water. In some burns, the four 54-L carboys were retained in the system to permit their simultaneous exposure to the phosphorus smoke downstream of the 760-L exposure chamber during selected experiments.

2.2.2 SGF No. 2 Fog Oil

The system used to generate fog oil vapors is illustrated in Figure 3. Fog oil was introduced at the rate of 5.8 mL/min by an FMI ceramic pulsatile laboratory pump into the fog oil vapor generator (Figure 4). The fog oil was flash-vaporized on contact with a 600°C vycor heater rod. Heat tape maintained the generator at 300°C downstream of the fog oil introduction port in order to control the rate of condensation of the newly generated fog oil vapor. A 4.0 L/min stream of nitrogen was used to flush the fog oil vapor from the generator into the dilution air stream. Nitrogen was used instead of air to eliminate the explosion hazard involved with generating a high concentration of fine oil vapor within an enclosed, high-temperature generator. The fog oil vapor and the air stream used to dilute the mixture was pulled through a standard aerosol exposure chamber by a transvector air flow amplifier at 10 ft³/min. Chamber air flow was monitored by measuring the pressure drop across an orifice in the chamber exhaust line.

The concentration of fog oil smoke in the chamber was determined using a GCA RAM-1 real-time aerosol monitor connected to an in-line TSI model 3302 100:1 diluter. Flow through the RAM/diluter combination was maintained at 5.0 L/min using the RAM's internal flowmeter. The aerosol monitor was calibrated to provide data on the fog oil vapor concentration by comparing the monitor's output obtained on an analog strip chart recorder to the results obtained gravimetrically using filter pad grab samples. Pre-weighed Gelman type AE 25-mm glass fiber filters were inserted into an open-faced filter holder positioned centrally inside the exposure chamber. Several 1- to 2-min grab samples were drawn at a flow rate of 1.0 L/min through the filters. The filters were then reweighed. Calibration data for the aerosol monitor were calculated by dividing the net fog oil mass on the filter by the volume of air pulled through that filter.

Four vaporizations of fog oil, three with Batch 1 fog oil (FO-1 through FO-3) and one with Batch 3 (FO-4) were conducted. The first vaporization, FO-1, was generated under 100% N₂. The other vaporizations, FO-2, FO-3 and FO-4, were generated in 4% excess air.

A total of 13 stainless steel, sterilized animal excreta collect pans were positioned inside the exposure chamber to catch the fog oil as it settled. In experiments FO-1 and FO-4, the chamber was allowed to reach maximum fog oil concentration, at which time the fog oil generator was turned off and the chamber sealed to allow time for the fog oil to settle onto the catch pans. Vaporization FO-1 consisted of five runs of approximately 15 minutes each, separated by an interval of approximately 1 hr to allow the more massive droplets to settle. Vaporization FO-4 consisted of

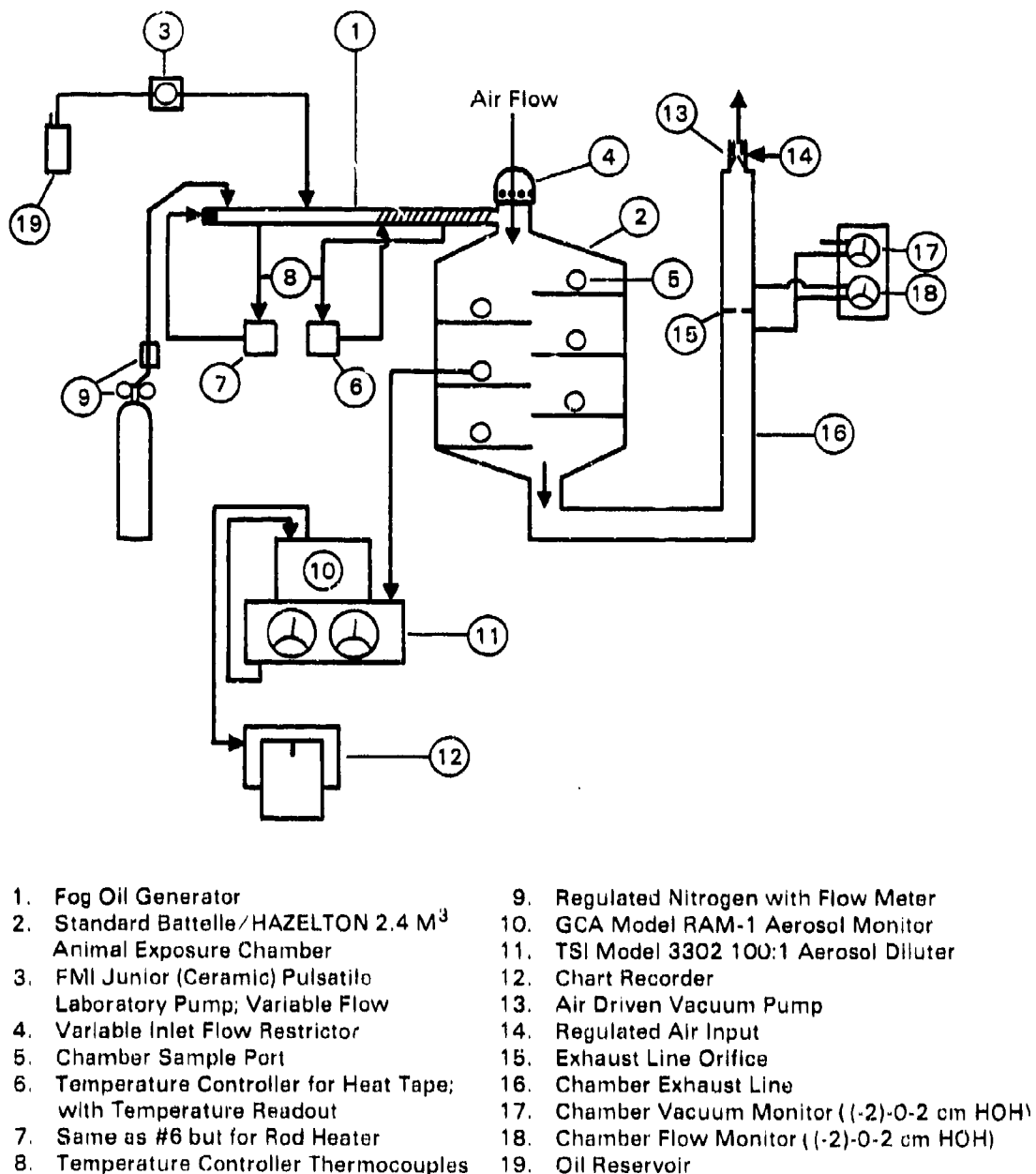


Figure 3. Fog Oil Vapor Generating and Collection System.

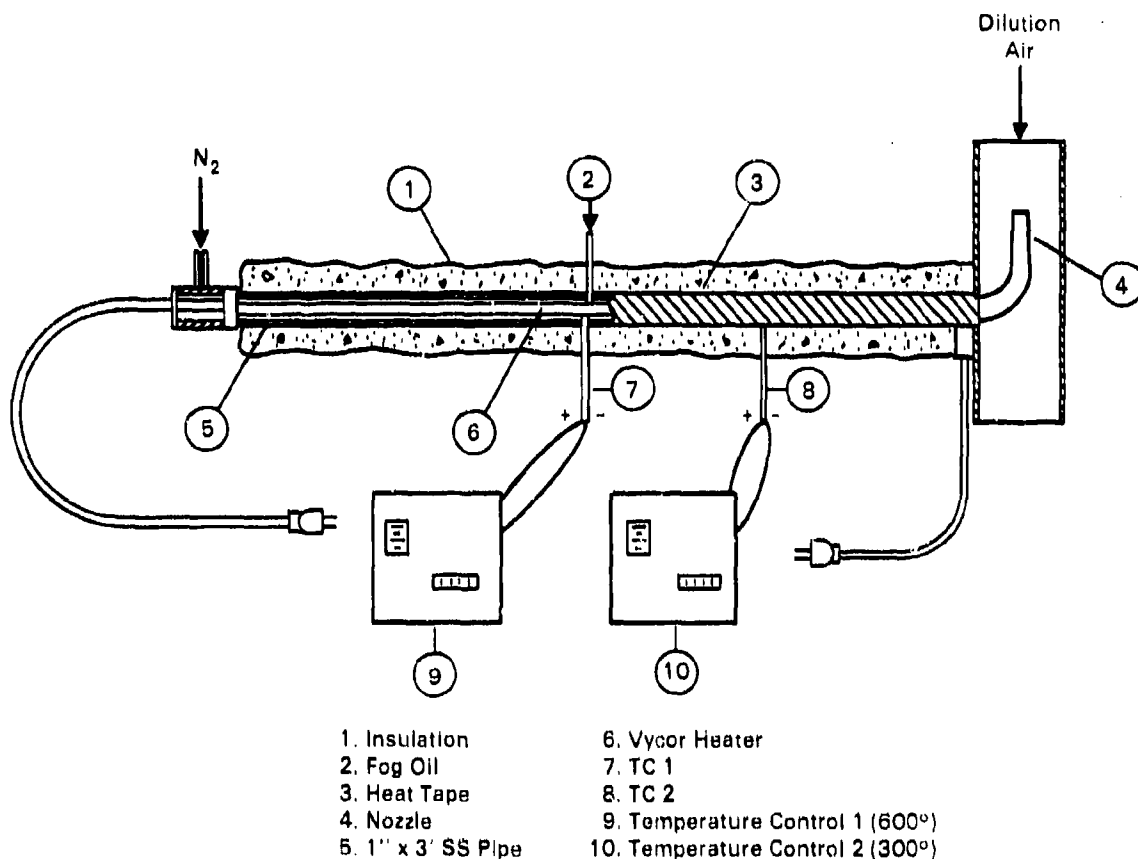
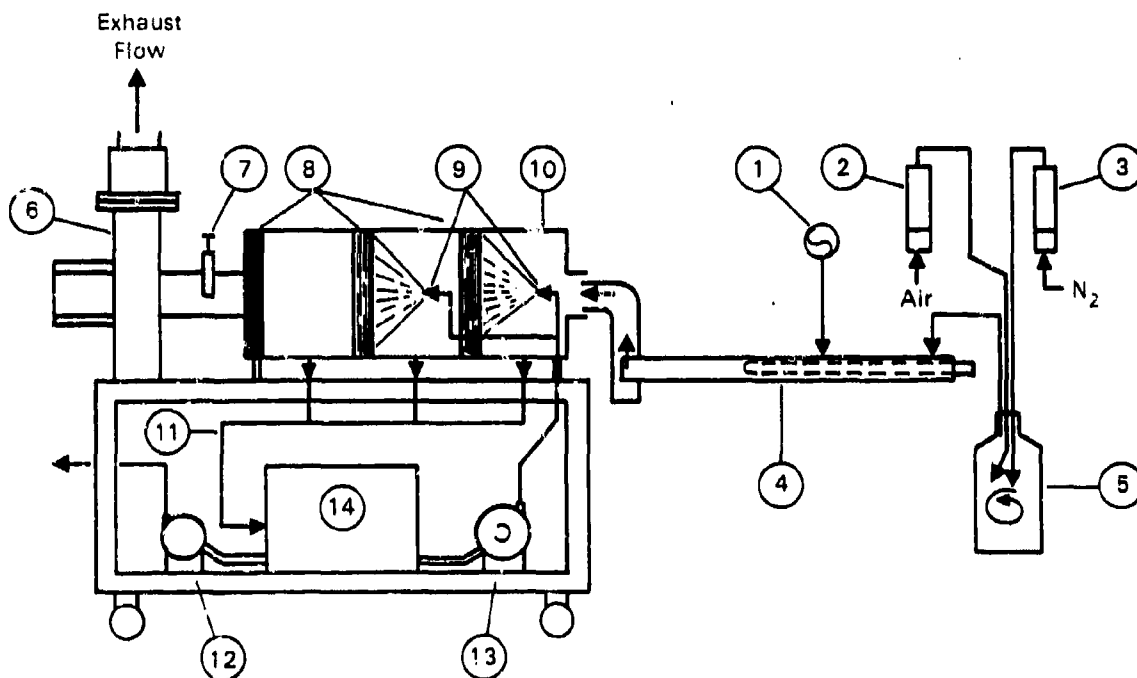


Figure 4. Fog Oil Vapor Generator.

11 30-min runs, each separated by at least 1 hr to allow for settling. At the end of each series of runs, the fog oil collected on the catch pans was scraped off with glass microscope slides (FO-1) or Teflon squeegees (FO-4) into volumetrically calibrated glass containers. A total of 80 mL of fog oil was collected from all 13 catch pans for experiment FO-1, and 1100 mL was collected for FO-4.

The system used to generate fog oil vapor for FO-2 and FO-3 is shown in Figure 5. Fog oil vapor was generated in the manner described for FO-1 but with minor changes. For these trials, 3.2 L/min of compressed air was mixed with 46.8 L/min of N_2 to provide approximately 4% excess air before the mixture entered the generator. The rate at which oil flowed into the



- | | |
|---------------------------------------|--------------------------------------|
| 1. Oil Metering Pump | 8. De-mister Pads |
| 2. Generator Air Rotameter | 9. Spray Nozzles |
| 3. Generator N ₂ Rotameter | 10. MYSTAIRE Laboratory Air Scrubber |
| 4. Fog Oil Generator | 11. Drain Lines |
| 5. Air-N ₂ Mixing Chamber | 12. Reservoir Drain Pump |
| 6. Blower Fan | 13. Spray Pump |
| 7. Airflow Control Gate | 14. Solution Reservoir |

Figure 5. Fog Oil Vapor Collection System with MYSTAIRE[®] Scrubber.

generator was reduced from 5.8 mL/min to 2.2 mL/min to lower the concentration of vapor and thus reduce the explosion potential caused when oxygen in the air was introduced into the fog oil generator.

Methods used to collect fog oil vapor in F0-2 and F0-3 differed from the catch pan settling methods used in F0-1 and F0-4. A MYSTAIRE[®] laboratory scrubber was used to pull the fog oil vapor from the generator in

[®] Trademark of the Heat Systems-Ultrasonic Inc., Framingdale, NY

experiments FO-2 and FO-3. This laboratory scrubber uses two fine mist sprays connected in series to efficiently (~90%) scrub particles from the air stream generated by a vacuum pump. In FO-2, the fog oil was collected in the recirculating scrubbing water for analysis. In FO-3, the fog oil was collected on a loosely packed glass-wool filter inserted between the fog oil generator and the MYSTAIRE® laboratory scrubber.

2.3 ANALYSIS OF PHOSPHORUS COMBUSTION PRODUCTS

Phosphorus combustion products were analyzed by two primary methods: inductively coupled plasma (ICP) emission spectrometry and ion chromatography.

2.3.1 ICP Analysis of Total Phosphorus

The total phosphorus in solutions generated by the burning of WP-BR or WP-F was determined with a Model 975 Jarrell-Ash (Division of Fisher Scientific; now Allied Analytical Systems) inductively coupled plasma (ICP) emission spectrometer. Aqueous samples were analyzed without modification (i.e., no acid added) and normally did not require filtration.

The Jarrell-Ash ICP uses two-point calibration. Normal setup uses 0.0 mg/L P (water blank) and a 10.0 mg/L P standard. An internal reference standard is not used since viscosity is not normally a problem with these samples. No on-peak spectral correction of any significance is made for P, though the actual program used is proprietary to Jarrell-Ash. No off-peak background correction is made. Drift in the ICP is controlled by running standards after approximately five samples. If the system has drifted any more than 3%, the instrument is recalibrated and the last sample is rerun. Precision is expected to be about ±50% at the detection limit (0.1 mg/L for P), ±10% at 5 times the detection limit, and ±3% at 50 times the detection limit.

In addition to P analysis, the ICP provided a scan of 32 other elemental concentrations simultaneously. This additional information was very helpful in monitoring solution preparations, in "debugging" smoke generation techniques, and in estimating the composition of precipitated material observed to form slowly in the harder waters.

2.3.2 Ion Chromatography

The lower oxyphosphoric-acid species were analyzed using a Dionex (Sunnyvale, California) model 16 Ion Chromatograph (IC) equipped with AG1 and AS1 separator columns and either a cation resin eluent-suppression column or a continually-regenerated fiber suppressor linked to a conductivity detector. Eluent was 3 mM NaHCO_3 plus 2.4 mM Na_2CO_3 . Phosphate and phosphite were resolved from other anions (F^- , Cl^- , Br^- , NO_3^- , SO_4^{2-}) in the water matrices. Detection limits were 0.02 mg/L for phosphate-P and 0.01 mg/L for phosphite-P. Hypophosphite co-eluted with F^- and any other non-retained conductive species in the void volume, and so could only be

estimated based on changes in the peak height for the samples versus the water blanks. Thus, detection of hypophosphite in reconstituted very hard water (Marking and Dawson 1973) would be approximately 0.03 mg/L as P, whereas in well water the detection limit would be no better than 0.05 mg/L as P.

Continued analysis of stock and exposure solutions resulted in a loss of column efficiency and an increase in back pressure. The back pressure was presumably caused by binding of the higher polyphosphates onto the column in the basic system. Reasonable column recovery was attained by back-flushing with a series of acid and base washes (0.1 N H_2SO_4 , 0.1 N NaOH, 0.2 N Na_2CO_3).

Calibration of the IC depends on comparison to known standard dilutions and, therefore, is limited by the accuracy of the dilutions. Sufficient standards are run to "bracket" the sample concentrations. No matrix effects were evident for phosphate or phosphite. Precision variation in tandem replicates was 1% for $\text{PO}_4^{3-}\text{-P}$ at 4.3 mg/L level and no worse than 8% for $\text{HPO}_4^{2-}\text{-P}$ at the 0.013 mg/L level.

Phosphate, phosphite, hypophosphite, and pyrophosphate solutions were prepared from reagent grade chemicals (MCB, Norwood, Ohio; MCB; Baker, Phillipsburg, New Jersey; and Fisher, Springfield, New Jersey respectively). Tripolyphosphate was supplied by Monsanto (St. Louis, Missouri). Tetrapolyphosphate, hexammonium salt, and the type 5, 15, and 25 polyphosphate glasses were obtained from Sigma Chemical Company (St. Louis, Missouri). Cyclic trimetaphosphate was prepared according to Ballou (1981). All other chemicals used were reagent grade.

2.3.3 Phosphorus Speciation

The separation scheme for polyphosphate speciation deviates slightly from the Yamaguchi et al. (1979) procedure. The procedure involves anion exchange using a high-salt eluent containing ethylenediamine tetracetic acid (EDTA) to complex any metals in the system, thereby slowing the depolymerization rate. The condensed phosphates are acid hydrolyzed and the resultant phosphate reacts to form a heteropoly blue complex with Mo(V-VI). Basic conditions used to separate condensed phosphates are listed in Table 2. Satisfactory results were obtained using a water bath (94°C) to heat both the acid hydrolysis and the color development loops.

In initial work, we used Na_4EDTA eluents (pH 7.5) to increase the retention times of the linear polyphosphates. Under this system, however, 3P and 4P coeluted. Subsequently, we returned to the Yamaguchi et al. (1979) eluent system containing Na_4EDTA (pH 10). Using their system, we observed up to 23 linear polyphosphates and 3 metaphosphates in the samples we processed.

Under the high performance liquid chromatography (HPLC) conditions given, it is not likely that phosphite and hypophosphite would be resolved

TABLE 2. CONDITIONS USED IN SEPARATION OF LINEAR AND CYCLIC
CONDENSED PHOSPHATES

HPLC Conditions:

Resin - Hitachi #2630 (a spherical anion exchange resin)
Column - 10 x 1 cm
Eluent A - 0.22 M NaCl + 5 mM Na₄EDTA (pH 10)
Eluent B - 0.53 M NaCl + 5 mM Na₄EDTA (pH 10)
Gradient - Curve 5, Waters 660 solvent programmer
Flow rate - 1.1 mL/min

Colorimetric Conditions:

Sample stream splitter percent = 30
Acid hydrolysis - 0.32 mL/min 3.45 M H₂SO₄
Aerosol 22 Solution - 0.10 mL/min 0.05% solution
Resample rate - 0.42 mL/min (~57% of hydrolysis mix)
Molybdate Solution - 0.32 mL/min 8.1 mM (NH₄)₆ Mo₇O₂₄ · 4H₂O in 0.6 M
H₂SO₄
Ascorbic Acid Solution - 0.23 mL/min 0.1 M ascorbic acid in 5% (V/V)
acetone plus 0.05% aerosol 22 solution
Detection - 660 nm

from phosphate. Even if they could be resolved, however, the phosphite and hypophosphite would not be oxidized to phosphate in the hydrolysis scheme and, consequently, would not be detected colorimetrically. Ultraviolet (UV) enhanced oxidation prior to acid hydrolysis would eliminate the detection problem, but the high concentration of chloride in the column effluent would slow the oxidation process. Since the ion chromatographic procedure is much faster and the inorganic phosphorous species are better resolved, it has remained the principal analytical method for the bulk of the samples. In practice, hypophosphite has been insignificant (<0.1%) and will not be reported. Although considerable improvements in the time required for HPLC analysis have been made by Brazell, Holmberg and Moneyhun (1984), resolution of the metaphosphates and of the higher polyphosphates (>13P) was somewhat diminished in the faster procedure. Work in this project was conducted using the slower Yamaguchi method only.

2.3.4. Other Analytical Techniques

Solvent extractions of WP-F stock solutions were performed, with slight variation, according to the method described by Lai (1979). Based on data in that report, extraction of white phosphorus into benzene (10:1 water to benzene ratio) was expected to be about 93% complete in 1 hr, and 100% in 3 hr; hydrolysis and back extraction into 8 M HNO₃ (10:1 benzene to acid ratio) would be 99% complete in 1 hr.

Initial extractions were performed using 10:1 and 5:1 extraction ratios for 1 to 3 hours, followed by back extraction at a 10:1 ratio with 6 M HNO_3 . Hydrolysis-evaporation of the benzene layers after back extraction showed no detectable remaining P by ICP analysis (equivalent to 0.02 mg/L P detection limit in original aqueous phase). Efficiency of the initial benzene extraction step could not be otherwise checked, since no GC procedure had been established for WP analysis. We assumed efficiency was similar to that quoted by Lai (1979).

Later extractions were modified to a 20:3 water to benzene ratio with contact for 2 hr, followed by water rinse and a 10:1 benzene to 6 M HNO_3 ratio and contact for about 1.5 hr.

A brief study of feasibility of thin layer chromatography (TLC) applications was based on methods of Ballou (1981) and Katz et al. (1981). Results of the TLC study were less than satisfactory, due to both extended operator time and lack of clarity in the resulting plates. Therefore, the TLC methods were discontinued from routine use.

Titration of the acid endpoints was done using a system composed of REC80 servograph, REA161 titrigraph module, REA260 derivative unit, ABU80 autoburette, TTT80 titrator, and PHM82 pH meter (Radiometer, Copenhagen). The first acid inflection was corrected for the partial neutralization caused by the carbonate content of the sample. The titration procedure indicated the presence of polyphosphate and that the species could not be entirely metaphosphates. Further prediction of the species in a mixture would have been little better than conjecture in the absence of actual speciation, so the titration procedure was not continued on a routine basis.

2.4 ANALYSIS OF SGF NO. 2 FOG OIL

This section describes the methods used to analyze bulk fog oil and to monitor oil-water mixtures (OWM) of fog oil. High performance liquid chromatography (HPLC) and analysis of total organic carbon were briefly investigated for monitoring oil-water dispersions (see Section 3.4).

2.4.1 Infrared Analysis of Total Oil

Standard solutions of SGF No. 2 Fog Oil at various concentrations were prepared in carbon tetrachloride (CCl_4). Infrared absorbance of these solutions was measured at 2927 cm and a calibration curve of absorbance versus concentration was prepared. Carbon tetrachloride was used to extract fog oil from acidified oil-water mixtures (OWM), and the resulting extracts were analyzed for infrared absorbance (IR) at 2927 cm. Least squares linear regression analysis of the calibration data yielded a linear fit ($R^2 = 0.997$) with the equation $Y = 0.0014 + 0.0023 X$. The concentrations of fog oil in each OWM- CCl_4 extract were determined by comparing the IR absorbance of the extract with the corresponding absorbance and fog

oil concentrations on the calibration curve. Concentrations of oil in the aqueous samples were then calculated by applying concentration factors accounting for volume differences between original aqueous samples (500 mL) and CCl_4 extracts.

Extraction efficiency of CCl_4 was indirectly determined by comparative studies of the IR absorbance of fog oil dissolved in CCl_4 . The IR absorbance was determined in 10 mL volumes of the fog oil CCl_4 solutions. The 10-mL fog oil- CCl_4 solutions were then mixed with 500 mL of acidified distilled H_2O to simulate the extractions of fog oil from exposure solutions. IR absorbance indicated a recovery of $103\% \pm 2.7\%$. A Beckman Acculab infrared spectrophotometer was used for all measurements. Performance of the spectrophotometer was checked before each use with a standard made of polystyrene film.

2.4.2 Gas Chromatography

High resolution gas chromatography (GC) with flame ionization detection (FID) was used to measure saturated and aromatic hydrocarbon and phenol concentrations in bulk oil and in OWMs of SGF No. 2 Fog Oil. Bulk fog oil samples were diluted with hexane to a concentration of about 10 mg/mL. One-liter volumes of OWMs were acidified and extracted twice with two 25-mL volumes of methylene chloride. The two extracts were combined, reduced to 1 mL under nitrogen, and the methylene chloride was exchanged for hexane. Saturated hydrocarbon fractions and aromatic hydrocarbon fractions were isolated from other fog oil components by passing the oil-in-hexane solutions through a 25-cm-long by 1.25-cm-diameter glass column packed with Davidson 100 to 200 mesh silica gel that had been activated at 150°C (Warner 1976). Saturated hydrocarbons were eluted with 40 mL of hexane. The aromatic fraction was then eluted with 87 mL of 20% methylene chloride in hexane. Both fractions were reduced in volume under nitrogen to approximately 1 mL for GC analysis.

Hydrocarbon components were quantified using the internal standard method. The gas chromatograph was calibrated by determining detector response factors for concentrations of known saturated and aromatic hydrocarbon standards. These response factors were compared to the detector response for an internal standard added to both standards and samples. Hexamethylbenzene was used as a standard for aromatic hydrocarbons and 2,6,10-trimethyldodecane was used for saturated hydrocarbons.

The fog oil and OWMs were analyzed for phenols. Phenols were extracted from oil samples into 0.1 N NaOH and acetylated by addition of acetic anhydride to the aqueous extract. Phenols present in OWMs were acetylated with acetic anhydride after a concentrated NaOH solution had been added to give a final concentration of 0.1 N NaOH. Phenol acetates were extracted from aqueous solution into 4 mL of hexane. Hexane extracts were concentrated and amended with n-dodecane as an internal standard. Detector response factors were determined for authentic phenol standards

amended to 0.1 N NaOH, acetylated, and extracted by the same method used for samples.

A Hewlett-Packard model 5840 or 5880 gas chromatograph fitted with a 60-m DB-5 fused silica bonded capillary column (J & W Scientific, Inc.) was used for GC analysis. Chromatographic conditions included split injection of a 2- μ L sample (10:1 split ratio). Following sample injection, the chromatograph oven temperature was maintained at 65°C for 4 min and then programmed to increase at 4°C/min to 260°C with a 20 min hold at 260°C.

2.4.3 Photolysis of Fog Oils

Batch 1 bulk and vaporized (FO-4) fog oils were used in the photolysis studies. Preliminary experiments were designed 1) to determine the optimum conditions for irradiation, and 2) to prepare photolyzed material for preliminary chemical and toxicity testing. Two samples of Batch 1 fog oil one vaporized (FO-4), and the other bulk, were tested. Photolysis was conducted in a flat-bottomed circular quartz container (74-mm diameter) covered with a quartz plate and kept at 20°C with a cooling plate. The quartz was transparent to light of wavelength 200 to 800 nm. A solar simulator (Oriel® model 6720), fitted with a filter to match the solar spectrum of light striking the earth at 90 degrees, was adjusted using a pyranometer such that the intensity striking the surface of the exposed oil was equal to 1.36 kW/m², the maximum intensity of sunlight striking the earth surface. Five milliliters of oil, forming a 0.116-cm layer in the apparatus, were exposed for each experiment.

As exposure of oils continued, the course of the reaction of the oil was monitored by visual inspection and by determining the number of free radicals generated by the radiation. Free radicals were monitored with a Varian electron-spin resonance spectrometer that had been calibrated with the stable free radical α , α' -diphenylpicrylhydrazyl.

2.4.4 Analysis of Photolyzed Fog Oil

Generally, the methods used to analyze photolyzed fog oil and OWMs prepared from photolyzed fog oil were similar to the methods described previously. Both UV and GC analyses were slightly modified, however.

UV Analysis

Ultraviolet absorbance spectra were determined for hexane extractions of the photolyzed oils. Stock solutions (OWMs) were extracted with iso-octane for analysis of total oil. A Beckman DB-25 spectrophotometer was used to monitor and record UV absorbance.

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GC Analysis

A 30-meter x 0.25 mm DB-5 nonpolar bonded phase capillary column (J & W Scientific) was used to separate water soluble components in the photolyzed OWM by GC. The instrument was linked to a Hewlett-Packard Model 5970A mass-selective detector to identify selected components.

2.5 CULTURE AND CARE OF TEST ORGANISMS

This section discusses characteristics of the dilution water used during Phases I and II of the project and the methods used to culture test organisms.

2.5.1. Dilution Water

Three sources of dilution water were used in this project. During Phase I, tests were performed with D. magna cultured in reconstituted distilled water (Marking and Dawson 1973) and well water. Columbia River water was used for Phase II tests with fish and invertebrates.

Three levels of reconstituted water were prepared (Table 3) from dilutions of the very hard formulation. Basic water chemistry parameters of well water and Columbia River water were determined bimonthly (Table 4). Assayed constituents of the dilution waters are listed in Table 5.

2.5.2. Fish

Four species of fish were used in toxicity tests: rainbow trout, fathead minnow, bluegill sunfish, and channel catfish (Table 6). Rainbow trout were raised at PNL facilities. The remaining three species were purchased offsite. Multiple purchases of fathead minnow and bluegill sunfish were necessary because of disease.

Stocks of fish purchased offsite were routinely quarantined in well water for a period of at least 10 days prior to acclimation in Columbia River water and testing. The fish were quarantined to minimize potential outbreaks of disease that could result from handling stresses that occur during shipment. The lone exception to this practice was a shipment of bluegill sunfish obtained in December 1984; these fish were acclimated directly to river water when they arrived. After the quarantine period, test fish were acclimated to river water and the selected test temperature for at least 7 days before testing.

Photoperiod

Stocks of fish were maintained on a 16-hr light to 8-hr dark photoperiod. Lighting was provided by a combination of incandescent (100

TABLE 3. MARKING AND DAWSON (1973) RECONSTITUTED FRESHWATER

Name	NaHCO ₃	CaSO ₄ ·2H ₂ O 2H ₂ O	MgSO ₄	KCl	pH ^a	pH ^b	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃
Very soft	12	7.5	7.5	0.5	6.8	6.4 - 6.8	10 - 13	10 - 13
Soft	48	30.0	30.0	2.0	7.4	7.2 - 7.6	40 - 48	30 - 35
Hard	192	120.0	120.0	8.0	8.0	7.6 - 8.0	160 - 180	110 - 120
Very hard	384	240.0	240.0	16.0	8.1	8.0 - 8.4	280 - 320	225 - 245

^a Approximate equilibrium pH after aeration.

^b Approximate equilibrium pH after aeration and with fish in water.

TABLE 4. BASIC WATER CHEMISTRY OF COLUMBIA RIVER WATER AND WELL WATER

Date	Well Water				Columbia River Water			
	pH	Hardness ^a	Alka- linity ^a	Conduc- tivity	pH	Hardness ^a	Alka- linity ^a	Conduc- tivity
12/06/83	7.6	119.4	121	306	NA ^b	NA	NA	NA
12/28/83	7.65	116.6	122	257	7.6	65.8	61.5	123
02/10/84	8.0	113.7	110	320	7.8	66.7	62.0	171
02/29/84	7.95	118.8	117	325	NA	NA	NA	NA
03/29/84	7.86	127.4	113	290	NA	NA	NA	NA
04/01/84	8.04	127.4	113	290	NA	NA	NA	NA
05/11/84	7.96	127.4	115	c	NA	NA	NA	NA
08/28/84	7.98	133.3	123	290	7.98	59.8	57.0	167
11/02/84	7.24	122.5	101	277	7.13	64.7	46	123
11/09/84	NA	NA	NA	NA	7.3	63.7	d	145
01/4/85	NA	NA	NA	NA	7.8	80.7	d	106
01/21/85	NA	NA	NA	NA	7.7	NA	65	128

^a mg/L as CaCO₃ equivalent.

^b Tests with this dilution water were not conducted at this time.

^c Instrument failed.

^d Values suspected to be in error due to faulty preparation of titrant.

TABLE 5. MEASURED CONCENTRATIONS (MG/L) OF COMPONENTS IN DILUTION WATER USED IN TOXICITY TESTS

Component	Columbia River Water	331 Well Water	Reconstituted Waters ^(a)			Algal Media ^b
			Very Hard	Hard	Soft	
F ⁻	(<0.01)	0.4	(<0.02)	(<0.02)	~0.01	(<0.01)
Cl ⁻	6.6	11	7.8	4	1.3	7.8
NO ₃ ⁻	19.4	12	(<0.03)	(<0.03)	(<0.04)	23.1
PO ₄ ³⁻	0.3	0.1	(<0.03)	(<0.03)	(<0.04)	(<0.2)
Br ⁻	(<0.05)	(<0.06)	(<0.03)	(<0.03)	(<0.04)	(<0.04)
SO ₄ ²⁻	10.6	~27	270	175	50	12.4
Ca	1.1	40	60	29	7.8	1.1
Na	11.3	19	100	61	18.4	14.1
Mg	4.0	7.7	32	25	7.4	4.6
K	(<0.3)	5.0	9.3	2.7	1.1	(<0.3)
Si	0.05	14	0.05	0.1	0.03	1.5
B	0.04	0.03	(<0.01)	(<0.01)	(<0.01)	0.18
Ba	(<0.01)	0.03	(<0.01)	(<0.01)	(<0.01)	(<0.01)
Sr	(<0.002)	0.16	0.08	0.01	0.01	(<0.002)
(Cd, Cr, Co, Ni, Zn)	(<0.02)	(<0.02)	(<0.02)	(<0.02)	(<0.02)	(<0.02)

^a Prepared by methods of Marking and Dawson (1973).

^b Prepared without added phosphate (Miller et al. 1978).

watt) and fluorescent (35 watt Sylvania Supersaver II or Cool White tubes) lighting. The incandescent lights were gradually phased on and off at the beginning and end of the photoperiod.

Feeding

Fish were fed dried, commercially prepared food (Silvercup, Murray Elevator, Murray, UT) two to three times daily. Bluegill sunfish fry obtained in September 1984, initially refused to feed. Feeding was induced by supplementing the dried swim-up size food with live Artemia larvae, Daphnia, and periphyton communities collected onsite. The mortality rate in the group of fish two weeks preceding the test was 22 out of more than 280 individuals. Mortalities were restricted to the smallest fish in the population. The shipment of bluegill sunfish obtained in December 1984 also refused to feed after arrival. Feeding was induced with frozen brine

TABLE 6. GENERAL INFORMATION ON FISH USED DURING PHASE II
TOXICITY TESTS

Species	Source	Range In Wt (g)	Age	Test Temp.
Rainbow Trout <u>Salmo gairdneri</u>	Pacific Northwest Laboratory Richland, Washington	0.08 - 0.69 ^a 0.05 - 0.15 ^b	<3 mo. <3 mo.	12°C 12°C
Fathead Minnow <u>Pimephales promelas</u>	D. Fattig ^a Brady, Nebraska ^b	0.23 - 1.21	<1.0 y	17°C
Bluegill Sunfish <u>Lepomis macrochirus</u>	D. Fattig ^c Brady, Nebraska	0.05 - 0.15	<1.0 y	17°C
	Osage Cat Fisheries ^a Osage Beach, Missouri	0.06 - 0.22	<1.0 y	17°C
Channel Catfish <u>Ictalurus punctatus</u>	D. Fattig Brady, Nebraska	0.79 - 2.16	<1.0 y	17°C

^a Fish used for WP-R toxicity test.

^b Fish used for RP-BR toxicity test; fish brood just started feeding when tested.

^c Fish used for RP-BR toxicity test.

shrimp obtained from a local pet store. No feeding problems were encountered with shipments of fathead minnow, channel catfish, or rainbow trout.

Disease

Two shipments of fish purchased for this project were destroyed because of persistent disease problems: the initial batch of fathead minnow purchased in July 1984 and a shipment of bluegill sunfish purchased in November 1984. There were no problems with disease in stocks of channel catfish or rainbow trout used in the project.

2.5.3. Macroinvertebrates

Four species of aquatic macroinvertebrates were used in this project (Table 7). The culture of each species of invertebrate was unique.

TABLE 7. GENERAL INFORMATION ON MACROINVERTEBRATES USED DURING PHASE II TOXICITY TESTS

Species	Source	Size	Age
<u>Daphnia magna</u>	Pacific Northwest Laboratory Richland, Washington	First Instar	12±12 h
<u>Hyallela azteca</u>	Environmental Protection Agency Corvallis, Oregon	>3 mm	Mixed
<u>Paratanytarsus dissimilis</u>	Environmental Protection Agency Duluth, Minnesota	--	7- 11 day
<u>Hexagenia</u> sp.	Rhodes Live Bait Amherst, Wisconsin	0.12-0.47 g wet ^a 0.37-0.88 g wet ^b	Mixed

^aTested in November and December 1985.

^bTested in January 1985.

Daphnia magna

Daphnia magna were cultured by static renewal. The cultures were fed the green alga Selenastrum capricornutum (see Section 2.4.3) and suspensions of blended trout chow. The algae were fed daily at a final concentration of 10⁸ cells/L and the trout chow was administered at a level of 5 mg/L after water exchanges. Water exchanges were performed three times per week. The feeding of trout chow was curtailed when it appeared that feeding may have been related to culture problems with a pathological infestation of fungus. Feeding of trout chow was resumed after the problem was alleviated.

Cultures were routinely maintained on well water or river water for this project. Special tests in Phase I of the project required acclimation of D. magna stocks to three levels of reconstituted water (Table 3). Culture water was changed and cultures were thinned three times a week. Cultures were grown at 20 ± 1°C on a 16:8 hr light:dark cycle.

First instars were collected for testing by isolating brood bearing females in a 500-mL beaker one day prior to testing. Offspring born overnight were used for testing on the following day.

Hexagenia

Mayfly larvae were maintained under flow-through conditions (~1.0 L/min;) in well water at 11.5°C to retard maturation and emergence.

Cultures were acclimated to river water (~100 mL/min; $17^{\circ} \pm 1^{\circ}\text{C}$) at least five days prior to testing. The photoperiod was 16:8 light:dark. The holding aquaria contained 4 to 5 cm of mud collected from the Columbia River. The larvae fed on naturally occurring detritus in the mud and periodic additions of rabbit pellets, dried oak leaves and suspensions of Cerophyl[®], a dried preparation of rye cereal leaves.

Organisms were collected by gently resuspending the mud substrate and transferring the larvae by hand to a bucket of river water. Exposures were initiated by transferring larvae in groups of 10 from the bucket to the aquaria with a small fish net. Organisms that appeared injured or swam irregularly were discarded.

Paratanytarsus dissimilis

Midge larvae were grown under static conditions in 16 L battery jars and 190 by 100 mm crystalizing dishes. Egg cases were collected by isolating two to four newly-emerged females in scintillation vials with 2 mL of river water. After two days, the egg cases were transferred to the larger culturing vessels. Fifty milliliters of a Cerophyl[®] suspension that had been blended and strained through plankton netting was added to each container. The suspension was prepared by blending 20 mL dry volume Cerophyl[®] in 100 mL of distilled water for 3 min. The suspension was then passed through plankton netting before the solution was added to the culturing chamber. Cultures were maintained at 20° to 22°C on a 16:8 hr light:dark photoperiod. Cultures raised for test purposes were raised in the crystalizing dishes. These cultures were started 10 to 13 days prior to testing to ensure that larvae would be 7 to 11 days old at testing.

Organisms were collected by prodding the organism's case with a pipet and capturing the larvae as it swam out the other end. Larvae were collected by this method and pooled into a 250-mL beaker for allocation to exposure beakers on the day of a test. The larvae were transferred with a 0.6-mm diameter pipet.

Hyalalela azteca

Cultures of H. azteca were grown at $17^{\circ} \pm 2^{\circ}\text{C}$ on a 16:8 hr light:dark photoperiod in a 30-L glass aquarium receiving ~100 mL/min of filtered (75 μm) river water. Dried oak leaves were used as a substrate and were supplemented with commercially available pelleted rabbit food two to three times weekly. Additional dried leaves were added as needed. Tests were performed with randomly allocated juveniles (~2 mm) and adults. Organisms were collected with a 0.6-mm pipet on the day of testing and pooled into a 600- to 1000-mL beaker for allocation to exposure beakers.

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2.5.4 Algae

The unicellular green alga, Selenastrum capricornutum, was grown in two different media for two distinct purposes. Bold basal media (Nichols and Bold 1965) adjusted to a pH of 6.6 was used to culture large quantities of algae for feeding D magna. This media was supplemented with vitamins (Goulden et al. 1982, see Appendix B). Algae were grown at 23° to 26°C with illumination provided by "cool-white" fluorescent lighting at a flux of 60 Einstein's $m^{-2} s^{-1}$ (4304 lumens m^{-2}). Charcoal filtered aeration was provided to enhance growth.

Algae were grown in EPA media (Miller, Greene and Shiroyama 1978) for toxicity testing purposes. The media was adjusted to a pH of 7.5 ± 0.1 . Algal cultures were grown for at least 96 hr on this media prior to toxicity testing. Basic growth conditions were the same as described for Daphnia food with daily shaking in lieu of aeration. Algal stimulation tests required that phosphorus be eliminated from the media. Separate batches of media were prepared without K_2HPO_4 for RP-BR and WP-F algal growth stimulation tests. All algal media were filtered (0.45 μm) under positive pressure (5 to 10 psi) to lower background levels of particles for the Coulter counter and autoclaved (15 psi and 121°C) before use.

2.6 TOXICITY TEST METHODS

This section discusses the methods used to generate stock solutions containing fog oil and phosphorus-based obscurants. Methods used to test algae, invertebrates and fish are also described.

2.6.1 Preparation of Exposure Solutions

This section describes the methods used to prepare exposure solutions with obscurants. Combustion of phosphorus-based obscurants and vaporization of fog oil were discussed in Section 2.2.

Red Phosphorus-Butyl Rubber and White Phosphorus-Felt

The methods for burning RP-BR and WP-F materials and collecting their combustion products are discussed in Section 2.2. Depending on the amount of stock solution required for testing, two systems could be used to generate stock solutions: four 54-L (12-gal) plastic carboys or a 760-L (200-gal) plastic carboy.

For tests with algae and macroinvertebrates (except Hexagenia), four 54-gal plastic carboys containing 1.0 L of dilution water were dosed with smoke. After a 1.5-hr settling period, the sides were rinsed by rolling the carboys onto their sides and inverting them. The contents of each carboy were used to prepare exposure solutions for toxicity testing.

For tests with fish and Hexagenia, the larger 760-L Fusion® carboys were filled with 5 L of dilution water for dosing. After a 4-hr settling

period, the carboy was vented under building vacuum and the sides were rinsed with an additional 15 L of diluent. The stock solution was drained by a PVC ball valve located in the corner of the carboy.

Fog Oil

Exposure solutions of bulk fog oil and vaporized fog oil were prepared using slow-mix methods comparable to methods used for testing other complex organic liquids (Dauble et al. 1983). The mixing methodology was designed to provide an exposure solution that was not overly burdened with excessive amounts of insoluble fog oil. Mixing energy was limited to levels that would reasonably simulate mixing energies expected to occur in most natural aquatic habitats. The mixing energy was increased in some tests at the end of Phase II.

A stock solution was prepared by slowly mixing the fog oil for 4 hr at room temperature ($23^{\circ} \pm 2^{\circ}\text{C}$). A Heidolph® variable-speed stirrer was fitted with a Nalgene three-blade propeller for mixing. The propeller blades were positioned 2.5 cm below the surface of the water. Mixing was started after the fog oil had been added to the system. Two different systems were used. For algal toxicity tests, 100 mL of fog oil was added to 10-L volumes of sterile media and mixed at $174 \pm 10\%$ rpm in 5-gal Pyrex® carboys for 4 hr. The mix was allowed to settle for 1 hr. The stock solution was obtained by siphoning the water out of the carboy while taking care not to entrain any oil. This setup was also used with well water and river water to prepare stock solutions for testing smaller invertebrates.

A large system was needed to generate sufficient stock solution to expose fish and *Hexagenia* larvae. A 12-gal Pyrex® carboy was filled with 30 L of diluent and 300 mL of fog oil. The solution was mixed for 4 hr and allowed to separate for 1 hr. Concentrations of oil comparable to the 10-L slow mix solution could be achieved by increasing the rpm to $240 \pm 10\%$.

Photolyzed Fog Oil Tests

Two series of tests were performed with photolyzed fog oils. The first series of tests used fog oil that had been photolyzed for 24 hr; the second series used oil that had been photolyzed for 7 days. In each series, four types of Batch 3 fog oil were tested: bulk, photolyzed bulk, vaporized (FO-4), and photolyzed-vaporized fog oil (see Table 2). Each was used to prepare a stock solution for preliminary chemical characterization and toxicity testing with *H. azteca*.

The generation of stock solutions with photolyzed fog oil differed considerably from the methods used for bulk and vaporized fog oil because of the small amounts (~4 mL) of photolyzed fog oil available. Stock solutions were prepared in two different ways. In the initial tests with 24-hr

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photolyzed fog oil, a magnetic stirrer was set at 400 to 600 rpm with a 3.75-cm Teflon[®] stir bar for 2 hr. Approximately 3.2 to 4.0 mL of photolyzed fog oil was mixed with 400 mL of river water and allowed to settle for 1 hr after mixing. The resulting stock solutions were turbid, indicating that the oil-water dispersion had not completely separated into discrete oil and water fractions. These solutions were used to prepare two exposure solutions, 100% and 20%, for screening toxicity tests with H. azteca.

The method for generating stock solutions was further modified to produce much less mixing energy than was present with the magnetic stirrer method. The magnetic stirrer method also made it difficult to control rpm, thereby introducing more variability in the mixes. To alleviate this problem, we used laboratory stirring motors and three-blade propeller apparatuses to mix the solutions. These were the same devices used for the standard slow mixes in Phase II of the project, except that the volumes used in the mix were greatly reduced. The mixing energy was set at $174 \pm 10\%$ rpm. Two and one half milliliters of oil was added to 900 mL of river water and mixed for 2 hr. The mixture was allowed to separate for 1 hr before the oil-water dispersion was siphoned off to prepare exposure solutions. Five dilutions of the photolyzed bulk and photolyzed-vaporized fog oil stock solutions were prepared for testing. Two exposure solutions (20% and 100%) were prepared with the bulk and the vaporized Batch 3 fog oil. After the initial 48-hr test, the 100% exposure solutions for the photolyzed bulk and vaporized fog oil were retested by siphoning off and combining the water from each beaker. Care was taken not to transfer any of the residues floating on the surface into clean exposure beakers. Hyalella azteca were exposed to the solutions for 48 hr. The unphotolyzed 100% solutions of bulk and vaporized fog oil stock solutions were not tested because no toxicity was apparent in the initial test.

2.6.2 Fish

Static conditions were used for all 96-hr acute toxicity tests. The methods used were based on standard practices developed by the American Society for Testing and Materials (ASTM 1980). The standard test conditions were modified in tests where fish were too large for the standard carboy or when not enough stock solution was available for setting up the test (Appendix C). These modifications involved reducing the number of fish per aquarium and/or reducing the number of test concentrations.

Screening tests with fog oil were conducted with fathead minnow at 100% and 10% stock solutions prepared under standard slow mix conditions (see Section 2.6.1). The remaining species of fish were not tested with fog oil because the three batches showed no apparent toxicity.

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Temperature, dissolved oxygen, and mortality were monitored daily. Ammonia concentrations were measured at the end of the tests (Appendix C). Measurements were taken more often in tests where the biomass of fish per aquarium approached the load limit of 0.8 g/L. Hydrogen ion concentrations were monitored daily when WP-F or RP-BR was tested. Concentrations of toxicants were measured at the start of all toxicity tests and at the end of tests in aquaria containing live fish in 96-hr tests with phosphorus-based obscitants. Phase I tests have shown that total P concentrations were stable for periods to 96 hr. Routine daily monitoring of an aquarium was terminated after all fish in that aquarium had died.

2.6.3 Macroinvertebrates

The methods used for acute tests of macroinvertebrates are based on standard methods prepared by ASTM (ASTM 1980). Because the biology of these macroinvertebrates is unique, the handling and testing of these organisms are discussed individually. Generally, tests with fog oil were performed in glass containers, and tests with phosphorus-based obscitants were conducted in plastic beakers.

Daphnia magna

Daphnia magna were tested in Phases I and II of this project. First instar daphnids (12 ± 12 hr) were used to initiate all tests. Phase I tests used well water and three hardness levels of reconstituted water: very hard, hard, and soft (Marking and Dawson 1973). The duration of these tests was extended from 48 to 96 hr because a bimodal toxic response had been noted earlier in the project and was considered worthy of continued investigation. During Phase II, all testing was conducted in Columbia River water for a period of 48 hr. These five types of dilution water provided a broad spectrum of water quality conditions that allowed us to evaluate the acidification potential of the phosphorus-based obscitants.

Tests with D. magna were also performed during Phase I to ascertain the effect of aging on the toxicity and chemistry of WP-F and RB-BR combustion products. In these tests, dilutions of well water that had been dosed with smoke were prepared and allowed to age for up to 28 days. During this time, 96-hr tests were started at elapsed times of 0 days, 5 days, and 26(RP-BR) or 28 (WP-F) days. Mortality temperature, and pH were monitored during the 96-hr testing period. During each test at 5 and 26 (or 28) days, a fresh 40% solution was prepared from the original stock solution. The fresh 40% solution monitored potential changes in the toxicity of the more concentrated stock solution.

In conjunction with the Phase I tests, chemical analyses were performed to evaluate the speciation and rates of hydrolysis of WP-F and RP-BR combustion products in water (see Section 3.2).

Hexagenia

Mayfly larvae were tested in duplicate aquaria containing 10 L of exposure solution. The weight of these organisms ranged from 0.12 to 0.47 g and the mean weights for selected test groups ranged from 0.25 to 0.29 g. Test organisms were collected from holding aquaria by resuspending the mud substrate and gently transferring the organisms by hand to a plastic pail. Test organisms were added to the exposure aquaria in groups of 10 in the order of random assignment of treatments. Observations for mortality, temperature, pH (phosphorus-based obscurants only), and dissolved oxygen were made daily for 48 hr. Test organisms that had been maintained on well water were acclimated to river water at least 5 days before testing. The organisms were tested at two temperatures. The screening toxicity tests with the three batches of fog oil were conducted at $20 \pm 1^\circ\text{C}$. The remaining tests were conducted at $17^\circ \pm 1^\circ\text{C}$ to reduce the potential for emergence. The photoperiod was 16:8 light to dark.

Hexagenia was the test organisms used to screen the toxicity of fog oil in Phase II of the study. Stock solutions were prepared in 30-L quantities, and two exposure solutions (100% and 20%) were prepared in duplicate for testing.

Paratanytarsus dissimilus

Midge fly larvae were tested in 100-mL beakers containing 90 mL of exposure solution. Four beakers were assigned to each treatment, and the tests lasted for 48 hr. Twenty organisms were exposed at each concentration tested. Reagent grade sea sand (0.3 g per beaker) and Cerophyl® (5 mg per beaker) were added to each beaker as substrate to prevent cannibalism. Test temperature was $20^\circ \pm 2^\circ\text{C}$ and the photoperiod was 16:8 light to dark. Observations were made only at 48 hr because the organisms had to be individually accounted for by removing them from their tubes.

Hyallolela azteca

Methods for testing amphipods were the same as those for testing P. dissimilus, except that observations were made at 24 and 48 hr and a substrate was not placed in the exposure beakers.

2.6.4 Algae

The methods used to assess the toxicity of the obscurants were based on a draft standard practice under preparation by ASTM for conducting acute toxicity tests with unicellular algae. The basic procedure involves the preparation of at least five exposure solutions and a control (six treatments). Stock solutions were prepared with EPA media filtered at $0.22 \mu\text{m}$ (Miller, Greene and Shiroyama 1978). Stock solutions prepared with

fog oil were mixed in 10-L amounts with a standard slow mix ($174 \pm 10\%$ rpm). White phosphorous felt and RP-BR smokes were vented into 54-L Nalgene carboys (see Section 2.6.1) to dose algal media. The alga *S. capricornutum* was inoculated at a final concentration of 10,000 cells/mL into 125-mL flasks (three flasks per treatment) containing 25 mL of exposure solution. The Batch 2 fog oil test inadvertently received an inoculum, resulting in a concentration of 2×10^5 cells/mL. In some of the later tests, the inoculum was increased to 2×10^4 cells/mL to enhance biomass estimates at the end of the test and reduce variability. The flasks were placed in a randomized grid system prior to inoculation.

Tests were conducted at $24^\circ \pm 2^\circ\text{C}$ under continuous lighting of $60 \mu\text{ Einstein's m}^{-2} \text{ s}^{-1}$ ($4304 \text{ lumens m}^{-2}$). Illumination ranged $\pm 10\%$ over the exposure grid. Algal biomass measurements (cell counts) were determined daily over the 96-hr duration of the test by use of a Model ZN Coulter counter. At 96 hr, a sample from each flask was removed for chlorophyll analysis. In some tests, chlorophyll measurements were also taken at 48 hr. The analysis involved the extraction of chlorophyll with 90% acetone after rupturing the algal cells by grinding an aliquot collected on a glass filter pad (Lorenzen 1966). The chlorophyll extracts were analyzed for fluorescence with a Turner Model 111 fluorometer equipped with a 5-60 blue primary filter and a 5-64 red secondary filter. The instrument was calibrated against a Turner Model 112 at the College of Ocean and Fisheries Science, University of Washington, to determine the appropriate calibration constants for the window selection.

Because a major combustion product of WP-F and RP-BR is orthophosphate (PO_4^{3-}) the algal toxicity test procedure was modified to test for the algal stimulation potential of these obscurants. A separate batch of EPA media deficient in PO_4^{3-} was prepared for dosing with smoke. After dosing, the stock solution was separated into two portions. One portion was neutralized with sodium hydroxide to the original pH of the media and the other was left acidic. Dilutions were prepared with the acidic smoke-dosed media and the PO_4^{3-} -deficient media, yielding pH values of 5.0, 5.5, 6.0, and 6.6, and a control. Identical dilutions were prepared with the neutralized portion in order to separate the effects of pH from excess PO_4^{3-} . Because of the low buffering capacity of the EPA media (16 mg/L CaCO_3 equiv.), the actual concentrations of phosphorus combustion products were low. Exposure solutions were monitored for PO_4^{3-} and PO_3^{2-} by ion chromatography and the total-P levels of the stock solution were determined by ICP.

2.7 STATISTICAL METHODS

This section describes methods used to analyze toxicity data and algal growth response data.

2.7.1 Toxicity Data

Acute toxicity tests with fish and macroinvertebrates were analyzed using the trimmed Spearman-Kärber (SPEAR Program) method (Hamilton, Russo and Thurston 1977). Median lethal concentration (LC50) values and 95% confidence intervals were reported for pH and total P. The pH values were not transformed to molar concentrations because of the wide range of pH values tested. The wide range (five orders of magnitude) of hydrogen ion concentrations could not be handled with the existing program. The LC₅₀ values and confidence intervals for pH agree well with the total P values, and are considered close estimates of the true values.

Mortality of fish in replicate aquaria were analyzed individually when differences in the pH or total P concentrations within a treatment were noteworthy (i.e., greater than 40% difference between aquaria). With the exception of tests with D. magna, none of the tests with fog oil yielded a mortality rate greater than 35% at the end of the test. In tests with D. magna, the phenomenon of "floaters" (p. 96) compromised our interpretation of the data. Consequently, LC50 values were not determined for any of the toxicity tests with fog oil.

2.7.2 Algal Toxicity and Growth Stimulation Tests

The growth response of S. capricornutum was measured daily to evaluate algal toxicity. Sampling included daily cell counts and chlorophyll analysis at the end of the tests. On isolated occasions, chlorophyll samples were taken at 48 hr. Statistical analyses were performed on cell count data only. Mean chlorophyll values were not statistically analyzed because the data base for cell counts spanned the entire test and were, therefore, the more rigorous data set.

Differences between treatments (toxicant concentration) were tested with a repeated measures analysis of variance (ANOVA) where cell counts taken over time constituted the repeated measures. The BMDP VAX 1983 version (Dixon et al. 1983) was used to analyze all cell count data. When either the treatment or main effects interaction were significant, additional tests were performed to determine significant differences between treatment means. Differences between treatment means (simple effects) were tested separately at each sample time with an F test (Winer 1971). If the F test was significant for any sample time, individual treatment means for that time were compared using a modified least significant differences (LSD) procedure for multiple pairwise comparisons (Winer 1971). Scheffé's critical value for multiple pairwise comparisons was used for individual mean contrasts in all tests.

Analyses were also performed on individual treatment means without Scheffé's critical value. In these tests, the error rate was unknown and was assumed to be greater than 0.05. These tests are referred to as less conservative tests. Whereas these tests showed a greater number of

significant comparisons between treatment means, they should be viewed with caution because of the uncertainties in the error rate. At most, we are certain that tests which showed no significance were indeed not significant. These additional tests were conducted to gain a better understanding of trends in the data.

Analysis of the growth stimulation tests with phosphorus-based obscuration paralleled the analysis of the acute toxicity tests. A repeated measures analysis of variance was used with two treatment factors, total P and pH (neutralized and non-neutralized), and one repeated measure (cell counts). Simple effects tests were performed and individual cell means were contrasted at each sample period. Scheffé's critical value was used to protect against underestimating the error rate for multiple comparisons.

3.0 RESULTS

This chapter describes aerosol generation, chemistry and toxicity of RP-BR smoke, WP-F smoke, and SGF No. 2 fog oil generated during the project. The rationale for the selection of settling as the method of choice for generating stock solutions is presented for all three obscurants. Aerosol concentrations of P-based obscurants are reported and compared to the resulting concentrations of total P in stock solutions.

The chemistry of RP-BR and WP-F smoke introduced into water is assessed. The relationship between composition of the combustion products in water, acidification of the dilution water, and the resulting aquatic toxicity is evaluated.

Bulk and vaporized fog oil, and OWMs prepared from them, are characterized, and the selection of methods used to monitor these materials in water are described. The production of oxidation products induced by photolysis of bulk and vaporized fog oil is discussed. The toxicity of the bulk fog oil and the influence of vaporization and photolysis on toxicity is evaluated.

3.1 AEROSOL GENERATION

Various methods were evaluated for dosing water with phosphorus-based obscurants and SGF No. 2 fog oil. Phosphorus-based obscurants were burned in a flow through system that vented the smoke into carboys where it could settle into dilution water. Bulk fog oil was vaporized and collected on stainless steel trays in a laboratory animal exposure chamber. Stock solutions for toxicity testing were prepared by mixing vaporized and condensed fog oil with dilution water.

3.1.1 Red Phosphorus-Butyl Rubber and White Phosphorus-Felt

The concentrations of smoke generated for dosing water varied by as much as a factor of four depending on the requirements of the specific test. Total phosphorus concentrations in the stock solutions were used to estimate the settling and recovery efficiency of the carboy dosing system. The system for dosing water with four 54-L carboys yielded a consistent relationship between the monitored smoke concentration and the total P levels measured in the stock solution. The expected concentration of total P in the stock solution was estimated by adjusting the mean smoke concentration for the volume of the carboys and the percentage of P in the smoke derived from desiccated samples. It was assumed that the percentage of P in the smoke was 30% (Van Voris et al. 1985).

For RP-BR burns in which four 54-L containers were dosed, the ratios of total P (mg/L) in the stock solutions to the expected concentration of total P ranged from 0.34 to 0.58 (Table 8). In comparison, the ratios for WP-F

TABLE 8. SUMMARY OF RP-BR SMOKE GENERATION AND THE RELATIONSHIP BETWEEN SMOKE CONCENTRATION AND PHOSPHORUS CONTENT IN STOCK SOLUTIONS

Burn Number	Carboy Size (L)	Burn Duration (min)	Mean Smoke Concentration (g/m ³)	Total Smoke Passed Through Carboy (g)	Smoke In Carboy At End Of Burn (g)	Total-P Stock Solution (g/L)	Expected P In Stock Solution (g/L)	Ratio: Assayed to Expected P
RP 3	54 L	10	11.3	2,820	0.599	0.099	0.182	0.55
RP 4	54 L	10	11.0	2,750	0.583	0.101	0.175	0.58
RP 5	54 L	10	N.A.	--	--	0.093	--	--
RP 6	54 L	15	5.94	2,228	0.314	0.034	0.094	0.36
RP 7	54 L	15	9.79	3,671	0.519	0.075	0.156	0.48
RP 8	54 L	15	18.20	6,825	0.965	0.097	0.290	0.34
RP 9	54 L	15	7.98	2,992	0.423	0.069	0.127	0.54
RP 10	760 L	45	9.76	43,920	7.418	0.282	0.111	2.53
RP 11	760 L	45	13.88	62,460	10.549	0.514	0.158	3.25
RP 12	760 L	45	12.51	56,295	9.504	0.537	0.143	3.75
	54 L	15	12.82	4,807	0.679	0.060	0.204	0.29
	54 L	30	12.82	4,807	0.679	0.060	0.204	0.29
RP 13	760 L	30	7.10	26,696	5.396	0.148 ^b	0.081 ^b	1.83
RP 14	760 L	30	5.25	15,753	3.990	0.198 ^b	--	--
	760 L	30	11.01	33,030	8.368	--	--	--
	54 L	15	11.48	4,305	0.608	0.183	0.183	0.56
RP 15	760 L	60	3.24	19,440	2.462	N ^c	--	--
	54 L	30	3.72	2,790	0.197	0.065	0.059	1.10
RP 16	760 L	30	10.61	31,830	8.064	0.198	0.121	1.64
	54 L	15	11.63	4,361	0.616	0.069	0.185	0.37

^a Assumes desiccated smoke was 30% P.

^b Test redone, stock solution prepared by combining the contents of two 760-L carboys

^c Test not conducted.

ranged from 0.38 to 1.24 (Table 9) and were generally higher than those observed for RP-BR. The smaller percentage and more variable distribution of P in WP-F may account for the wider variation in the range of ratios for WP-F. A ratio of 1.00 indicates that all smoke in the 54-L carboys settled and was dissolved when the sides were rinsed. These ratios were consistently <1 in all burns of 17 min or less except for WP-8. Burns WP-2 and WP-3 were excluded because two rather than four carboys were dosed and the measured flow rates per carboy were 41 L/min instead of 25 L/min.

Toxicity tests with fish required larger volumes of stock solution than were required for tests with invertebrates. The larger volumes of solution generally contained greater quantities of combustion products. The relatively consistent relationship between expected and observed total P concentrations observed in the 54-L system does not hold up for stock solutions generated in the 760-L Fusion carboys. The latter ratios ranged from 1.73 to 2.34 for WP-F (Table 9) and from 1.64 to 3.75 for RP-BR burns (Table 8). Ratios in excess of 1.0 indicate that more P was found in the stock solutions than could be accounted for in smoke left in the carboy when the burn was terminated. These differences may in part be explained by increased amounts of combustion products adhering to the sides of the carboys as the smoke concentrations were brought to equilibrium. Burns in the Fusion carboys lasted 30 min and longer to allow the smoke concentration in the larger carboys to reach equilibrium. The Fusion carboys had a greater inside surface area (53,475 cm² compared to 7,428 cm² in the 54 L carboys) to which the smoke could adhere. Either of these factors could account for the higher than predicted concentrations of total P in the stock solutions.

Data from the 54-L carboy burns was used to establish the relationship between the amount of smoke necessary to create toxic conditions as indicated by total P and pH. Based on the mean of seven RP-BR burns, the ratio of total P in the stock solution to the estimated mass of smoke in the carboys at the time the burn was at equilibrium was 0.14 ± 0.03 . The ratio for WP-F, also based on seven burns, was 0.23 ± 0.08 . Comparison of expected to measured concentrations does not account for retention of smoke during the burn. Retained smoke would increase the ratio. It is possible that turbulence within the carboys can minimize the retention of smoke on the sides of the carboys during the burns. With a flow rate of 25 L/min, the volume of the carboys turns over every 2 minutes. In comparison, the turnover rate in the 760-L Fusion carboys is 7.6 minutes. Because of the large imbalance of assayed P to expected P in the stock solutions, caution should be used when attempting to extrapolate this relationship to field situations.

3.1.2 SGF No. 2 Fog Oil

Settling of fog oil after repeated burns (FO-1) was selected during Phase I as the best method of generating and collecting vaporized fog oil for Phase II tests (Table 10). The trial vaporization with the MYSTAIRE[®]

TABLE 9. SUMMARY OF WP-F SMOKE GENERATIONS AND THE RELATIONSHIP BETWEEN SMOKE CONCENTRATION AND PHOSPHORUS CONTENT IN STOCK SOLUTIONS

Burn Number	Carboy Size (L)	Burn Duration (min)	Mean Smoke Concentration (g/m ³)	Total Smoke Passed Through Carboy (g)	Smoke In Carboy At End of Run	Total-P Stock Solution (g/L)	Expected P In Stock Solution (g/L)	Ratio: Assayed to Expected P
WP 2	54 L	7	5.12	1.469	0.271	0.368	0.081	4.53 ^b
WP 3	54 L	8	5.44	1.801	0.288	0.380	0.086	4.40 ^b
WP 4	54 L	10	6.50	1.625	0.345	0.664	0.104	6.38
WP 5	54 L	10	8.60	2.150	0.456	0.101	0.137	0.74
WP 6	54 L	10	9.80	2.450	0.519	0.132	0.156	0.85
WP 7	54 L	15	11.27	4.226	0.597	0.120	0.179	0.67
WP 8	54 L	14	8.21	2.874	0.435	0.163	0.131	1.24
WP 9	54 L	12	7.33	2.199	0.388	N.A.		
WP 10	54 L	17	14.77	6.277	0.783	0.089	0.235	0.38
WP 11	54 L	15	8.86	3.323	0.470	0.125	0.141	0.89
WP 12	54 L	15	8.85	3.319	0.469	0.089 ^c	0.141	0.63
WP 13	760 L	44	5.80 ^d	25.520	4.410	0.588 ^c		
WP 14	760 L	30	3.51 ^d	--	--	0.447	--	--
	760 L	30	3.34 ^d	--	--	0.430	--	--
	760 L	30	3.34 ^d	--	--	0.430	--	--
	54 L	15	3.34 ^d	--	--	0.043	--	--
WP 15	760 L	30	20.82	62.460	15.820	0.410	0.237	1.73
	760 L	30	20.82	62.460	15.820	0.410	0.237	1.73
WP 16	760 L	35	20.53	71.855	15.600	0.548	0.234	2.34
	54 L	15	22.24	8.340	8.340	0.145	0.354	0.41

^a Assumes desiccated smoke was 30% P.

^b Flow rate per carboy was 41 L/min, all other 54-L carboy flow rates were 25 L/min.

^c Stock solution contaminated with tube condensate.

^d Filtering device was faulty, values were probably low.

TABLE 10. SUMMARY OF FOG OIL VAPOR GENERATIONS

Designation	Fog Oil	Vapor Concentration (g/m ³)		Volume of Fog Oil Vaporized (mL)	Fog Oil Generation Time (min)	Total Volume of Fog Oil Collected (mL)	Fog Oil Collected %
		x	SD				
FO-1	Batch 1	13.6	1.1	476	82	80	16.8
FO-2	Batch 1	a		255 ^a	120 ^a	230 ^a	90.0 ^a
FO-3	Batch 1	b		355	195	128	36.1
FO-4	Batch 3	12.0	1.3	1990	334	1100	55.3

^a Fog oil was vented from the smoke generator into a MYSTAIRES® laboratory scrubber that removed the oil from the air with two fine water mists connected in series. Estimated values are based on 90% collection efficiency and volume of fog oil vaporized.

^b Fog oil was exhausted from the smoke generator into a horizontal glass wool filter in line to the MYSTAIRES® laboratory scrubber.

scrubber (FO-2) produced a totally emulsified solution that failed to separate into a discrete oil-water dispersion suitable for toxicity testing. Collection of vaporized fog oil by filtration of the aerosol in glass wool (FO-3) was cumbersome, labor intensive, and had a low yield per unit effort. Moreover, adsorption of oxidized organics to the glass wool could alter the composition and resulting toxicity of the vaporized fog oil. Bubbling vaporized fog oil through water was assumed to be as inefficient as was observed with RP-BR burns (see Section 3.1.1). The negative aspects of aqueous scrubbing and glass wool filtration, coupled with the fact that settling represented the most realistic mode of vapor collection, dictated the selection of the settling method.

Vapor generations FO-1, FO-2, and FO-3 were conducted with Batch 1 fog oil. This was the oldest, darkest (possibly more oxidized) batch of oil. Batch 3 fog oil was used for FO-4 because there was no apparent difference in toxicity among the three batches of fog oil, the drum had not been opened prior to its arrival at PNL, and it was the most recently manufactured batch of oil.

In the first settling test (FO-1), fog oil vapor was generated with 100% nitrogen. In subsequent generations, the vapor was generated with a mixture of air (4%) and nitrogen, yielding an air stream of ~0.8% O₂. This percentage of O₂ represents the theoretical concentration of oxygen present in the exhaust of a finely tuned gasoline engine. Fog oil vaporized in the presence of ~1.2% O₂ was distinctly darker than fog oil generated in 100% N₂ and contained a higher concentration of free radicals than the original parent oil (see Section 3.3.3).

3.2 PHOSPHORUS-BASED OBSCURANTS

Combustion of RP-BR and WP-F materials produces a smoke containing many oxidation products of P. Introduction of these materials into water results in a lowering of pH and the addition of linear and cyclic oxyphosphoric acids. This section discusses the chemistry and resulting toxicity of these combustion products in water. Acute toxicity was related to decreases of pH caused by hydrolysis of the phosphorus combustion products.

3.2.1 Chemistry

This section reviews the procedural development of methods used to characterize phosphorus combustion products in water and to identify the composition and speciation of combustion products in water. This section also summarizes the basic characteristics of stock and exposure solutions used for aquatic toxicity testing.

Preliminary Studies

Initial tests to determine the best method of dosing water with phosphorus smoke showed that allowing smoke to settle under static conditions over water appeared to be more realistic of field conditions than allowing it to bubble through the water. Static settling resulted in a lower ratio of phosphate to total P and also a lower ratio of phosphite to phosphate. Static and bubbling procedures and the resulting changes in water composition are compared in Figures 6 and 7. The loss of buffering capacity (decrease in HCO_3^-) results from titration by the addition of phosphoric acids. Though phosphate concentrations increased with time, the change was not dramatic. Rinsing of the container walls following static settling greatly increased the level of total P in the solutions (Figure 6). Concentrations of PO_4^{3-} were re-examined after prolonged aging. After 70 days, phosphate accounted for only one third of the total P in the bubbled water test; between 150 and 180 days the phosphate level remained at roughly 50%. Based on these results, plus the fact that settling better typifies actual field conditions, the settling mode was selected for dosing water.

Combustion products of RP-BR and WP-F were similar. Precipitated material formed when smoke-dosed waters were diluted or neutralized. The critical pH level appeared to be about 6. Changes in soluble elemental composition of dilutions of RP-BR and WP-F smoke-dosed well water are shown in Tables 11 and 12, respectively.

Significant losses from the 40% dilution of each burn are further summarized on a molar basis in Table 13. The precipitated material approaches a 1:1 ratio of P to Ca. Although Sr losses are minor on a mass basis, they represent 21% to 57% of the initial soluble concentration. It is likely that Sr could be used as an early indication of precipitation before the event becomes visually evident. A fresh 40% dilution from WP-F well water stock more closely resembled the day 0 dilution, as expected, since the unneutralized stock solutions did not show signs of precipitation.

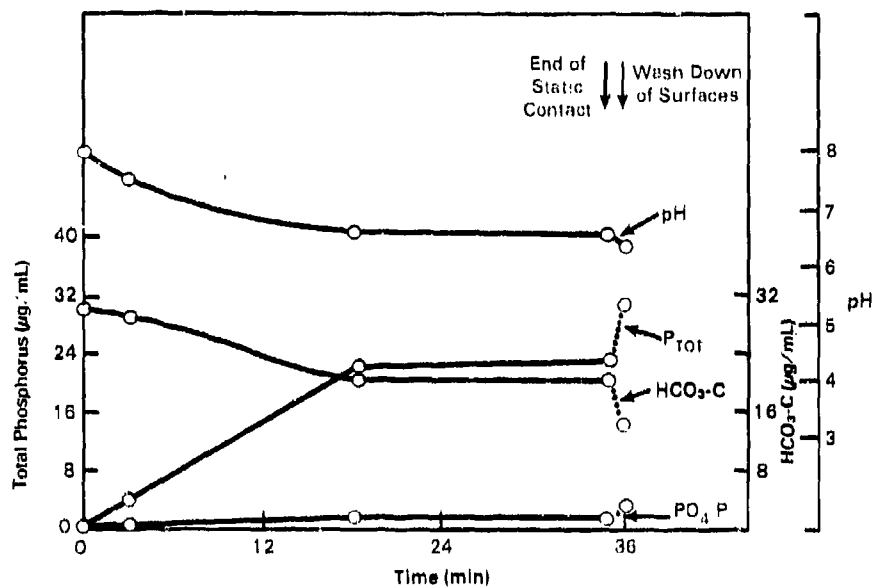


Figure 6. Change in dilution water following settling of RP-BR smoke (Burn RP-1).

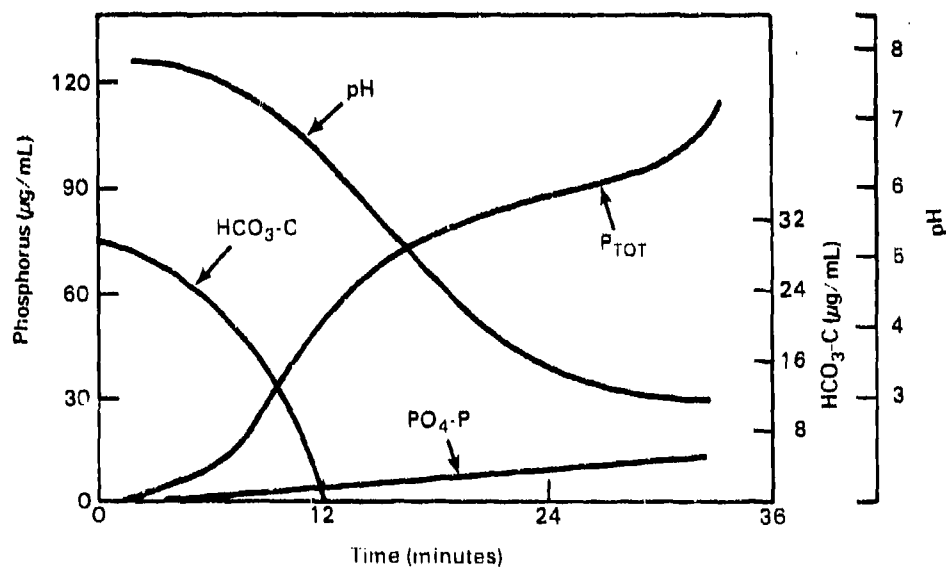


Figure 7. Effect of RP-BR smoke bubbled through well water (117 mg/L alkalinity).

TABLE 11. CHANGES IN SOLUBLE ELEMENTS IN WELL WATER SOLUTIONS,
ACCORDING TO AGE AND CONCENTRATION, FROM A RP-BR
COMBUSTION (BURN RP-4)

Solution	Age (days)	Elements (µg/mL)						
		P (0.1) ^a	Ba (0.002)	Ca (0.01)	K (0.3)	Mg (0.06)	Si (0.02)	Sr (0.001)
Stock	0	101	0.05	40.6	5.4	7.9	14.7	0.17
	5 ^b	101	0.02	41.6	5.5	8.0	14.7	0.17
	33	104	0.03	44.3	5.3	7.8	14.9	0.17
70%	0	73.6	0.02	40.5	5.5	8.0	14.7	0.17
	5	74.3	0.01	41.7	5.9	8.2	15.1	0.17
	33	71.0	0.02	42.6	4.9	7.9	14.4	0.16
50%	0	52.5	0.02	40.2	5.8	8.1	14.9	0.16
	5	47.2	0.002	37.9	5.9	7.8	14.1	0.13
	33	47.8	0.005	38.5	5.1	7.8	14.4	0.12
40%	0	42.4	0.01	40.3	5.9	8.1	15.0	0.16
	5	33.6	DL ^c	33.9	5.8	7.6	14.2	0.09
	33	30.7	DL	29.2	5.3	7.4	14.2	0.07
30%	0	31.5	0.01	40.3	5.8	8.1	15.0	0.16
	5	25.1	0.006	35.7	5.1	7.7	14.6	0.12
	33	19.5	DL	28.4	5.3	7.3	13.6	0.08
20%	0	20.4	0.02	40.1	5.5	8.0	14.7	0.16
	5	16.8	0.007	38.4	5.0	7.8	14.3	0.14
	33	12.0	DL	32.0	5.5	7.5	12.3	0.11
10%	0	11.2	0.13	40.3	5.6	8.0	14.8	0.16
	5	10.0	0.01	41.1	5.4	8.0	14.5	0.16
	33	8.5	0.01	39.1	5.3	7.9	13.4	0.14
5%	0	4.3	0.01	40.1	5.4	7.9	14.7	0.16
	5	4.5	0.01	41.9	5.2	8.0	14.5	0.16
	33	5.2	0.02	44.4	5.4	8.0	13.6	0.16
Well Water (Background)	0	DL	0.01	40.3	5.8	8.0	14.9	0.16
	5	DL	0.01	41.3	4.9	7.8	14.3	0.15
	33	DL	0.02	41.4	5.2	7.9	14.5	0.16

^a Detection limit.

^b Final analysis on 33-day-old solutions sampled at the (26-day) time of toxicity testing.

^c Below detection limit.

The breakdown of polyphosphates to phosphate was slow, as typified by the RP-BR and WP-F smoke-dosed well waters (Tables 14 and 15, respectively). The high percentage of $\text{PO}_4^{3-}\text{-P}$ for both RP-BR and WP-F waters peaks in the 50% dilution concentration. Precipitation is just becoming evident at that concentration and is heaviest, visually, in the 40% and 30% dilutions. The

TABLE 12. CHANGE IN SOLUBLE ELEMENTS IN WELL WATER SOLUTIONS, ACCORDING TO AGE AND CONCENTRATION, FROM A WP-F COMBUSTION (BURN WP-5)

Solution	Age (days)	Elements (µg/mL)						
		P (0.1) ^a	Ba (0.002)	Ca (0.01)	K (0.3)	Mg (0.06)	Si (0.02)	Sr (0.001)
Stock	0	101	0.01	41.2	5.0	7.7	14.1	0.16
	5	104	0.02	42.1	5.1	8.0	14.6	0.17
	28	101	0.02	41.2	4.5	7.8	14.2	0.18
70%	0	71.7	0.03	41.4	5.0	7.7	14.1	0.16
	5	71.7	0.01	41.6	5.3	7.8	14.2	0.18
	28	70.4	0.01	41.0	5.0	7.9	14.3	0.14
50%	0	51.1	0.02	41.1	5.2	7.7	14.1	0.16
	5	51.1	0.01	39.9	5.1	7.8	14.3	0.14
	28	48.9	0.01	38.0	5.0	7.8	14.2	0.12
40%	0	40.3	0.02	40.7	5.2	7.6	14.0	0.15
	5	37.0	0.01	35.6	5.0	7.5	14.1	0.11
	28	36.2	0.01	35.2	5.0	7.8	14.5	0.12
30%	0	30.0	0.02	40.4	5.2	7.7	13.9	0.15
	5	26.1	0.01	33.6	5.1	7.5	14.4	0.09
	28	24.9	0.01	33.5	4.7	7.6	14.6	0.11
20% ^(b)	0	20.0	0.01	40.5	5.1	7.6	14.0	0.15
	28	13.8	<0.01	32.8	4.8	7.6	14.6	0.10
10% ^(b)	0	9.8	0.01	40.6	4.9	7.6	14.1	0.15
	28	7.3	<0.01	36.1	5.0	7.6	14.2	0.11
Well Water Background	0	0.4	0.01	40.3	4.7	7.6	14.0	0.14
	5	0.2	0.01	39.1	4.8	7.7	14.3	0.14
	28	0.4	0.01	40.3	4.6	7.8	14.1	0.13
40% made fresh from stock on day of test	5	40.4	0.02	38.9	5.2	7.7	14.7	0.13
	28	41.6	0.01	40.6	5.2	8.0	14.5	0.13

^a Detection limits.

^b Twenty and 10% solutions were not analyzed on day 5.

drop in PO_4^{3-} -P percentage of soluble P, in the lower dilutions, does not necessarily indicate that the precipitating species are phosphates. The phenomenon suggests that concentration of phosphorus combustion products represents a critical factor in precipitation and may be related to the resultant pH.

Precipitation was dramatic upon neutralization and was followed by a gradual decrease in solubility of P and Ca (Table 16). The precipitated material was not fully characterized, but the observed changes were

TABLE 13. SIGNIFICANT LOSSES IN SOLUBILITY WITH TIME FOR
DILUTED (40%), NON-NEUTRALIZED 331 WELL WATER
ALIQUOTS

Element	mM Drop in Solubility	
	WP-F	RP-BR
P	0.13	0.38
Ca	0.14	0.28
Mg	(-0-)	0.03
K	0.004	0.015
Sr	0.0003	0.001
---	---	---
Initial P Total	1.30	1.37
Age (Days)	28	33

consistent with the formation of amorphous alkaline earth compounds and/or mixed salts of tripolyphosphates and pentapolyphosphates (Van Wazer 1958).

Hydrolysis of polyphosphates in RP-BR and WP-F smoke-dosed waters proceeded to phosphate slowly. Although long-term hydrolysis studies were outside the scope of this project, a number of stock solutions were periodically re-analyzed for phosphate to determine what portion of the polyphosphates had undergone complete hydrolysis. Since these observations were mainly for illustrative purposes, samples were merely capped and stored at room temperature between analyses; this handling of samples fails to provide the sterility and environmental control necessary for valid hydrolysis studies.

The results can best be described as a scatter of rates not correlated to water type, phosphorus type burned, or pH (neutralized versus non-neutralized). Such results illustrate the range of responses seen with aging and also emphasize the current lack of understanding of the mechanisms involved.

Several solutions from WP-F burns were analyzed for elemental P (i.e., P_4) using solvent extraction and ICP detection of P. Results usually were bordering on the ICP detection limit, but appeared to represent no more than 0.01% of the total P in solution.

TABLE 14. COMPARISON OF $\text{PO}_4^{3-}\text{-P}$ TO TOTAL P IN RP-BR SMOKE CONTACTED WITH WELL WATER, AS A FUNCTION OF SOLUTION AGE AND DILUTION

Dilution	Day	pH	Total P ($\mu\text{g/mL}$)	$\text{PO}_4^{3-}\text{-P}$ ($\mu\text{g/mL}$)	$\text{PO}_4^{3-}\text{-P}/$ Total P (%)
Stock	0	3.2	101	13.6	13.5
	5	3.3	101	14.6	14.5
	26	3.1	103	21.1	20.5
70%	0	5.1	73.1	9.7	13.2
	5	4.9	74.3	10.4	14.1
	26	5.1	71.0	15.2	21.5
50%	0	6.2	52.5	7.0	13.3
	5	7.0	47.2	7.8	16.6
	26	7.0	47.8	16.4	34.4
40%	0	6.6	42.4	5.6	13.1
	5	7.5	33.6	5.7	17.0
	26	7.5	30.7	7.7	25.2
30%	0	6.8	31.5	4.3	13.3
	5	8.0	25.1	4.1	16.3
	26	8.0	19.5	5.3	27.4
20%	0	7.0	20.4	2.7	13.1
	5	8.1	16.8	2.7	15.8
	26	8.2	12.0	3.6	30.0
10%	0	7.6	11.2	1.5	13.1
	5	8.3	10.0	1.5	15.2
	26	8.3	8.5	2.1	24.9
5%	0	7.6	4.3	0.6	14.3
	5	8.4	4.5	0.7	14.7
	26	8.4	5.2	1.4	27.7
Well Water (background)	0	7.9	(<0.1) ^a	.05	~100
	5	8.4	ND ^b	ND	ND
	26	8.3	(<0.1)	.03	~60

^aTotal P determined on freeze-dried concentration (0.05 $\mu\text{g/mL}$).

^bND = not determined.

TABLE 15. COMPARISON OF $\text{PO}_4^{3-}\text{-P}$ TO TOTAL P IN WP-F SMOKE CONTACTED WITH WELL WATER, AS A FUNCTION OF SOLUTION AGE AND DILUTION

Dilution	Day	pH	Total P ($\mu\text{g/mL}$)	$\text{PO}_4^{3-}\text{-P}$ ($\mu\text{g/mL}$)	$\text{PO}_4^{3-}\text{-P/}$ Total P (%)
Stock	0	2.9	101	15.0	14.9
	5	3.1	104	15.5	14.9
	28	3.0	101	18.8	18.6
70%	0	4.5	71.7	10.3	14.4
	5	4.7	71.7	10.8	15.1
	28	4.7	70.4	15.0	21.3
50%	0	5.8	51.1	7.4	14.5
	5	6.7	51.1	8.0	15.7
	28	6.9	48.9	20.2	41.3
40%	0	6.3	40.3	5.9	14.6
	5	7.6	37.0	6.0	16.2
	28	7.5	36.2	9.9	27.3
30%	0	6.6	30.0	4.4	14.7
	5	7.8	26.1	4.3	16.5
	28	7.9	24.9	5.9	23.7
20%	0	6.9	20.0	3.0	15.0
	5	8.1	ND ^a	2.6	ND
	28	8.2	13.8	4.3	31.4
10%	0	7.3	9.8	1.5	15.7
	5	8.3	ND	1.6	ND
	28	8.4	7.27	2.3	31.6
Well Water (background)	0	8.0	0.4	0.04	~11
	5	8.5	0.23	0.05	~22
	28	8.5	0.41	0.04	~10

^aND = Not determined.

TABLE 16. PRECIPITATION FROM NEUTRALIZED WELL WATER (117 MG/L ALKALINITY) AND RECONSTITUTED VERY HARD WATER (230 MG/L ALKALINITY) AFTER CONTACT WITH WP-F SMOKE

Losses of Elements	Water	
	Well Water	Very Hard Water
Initial (<1 day)		
P	0.30	0.57
Ca	0.22	0.56
Gradual (1-6 days)		
P	0.14	0.05
Ca	0.14	0.06

Speciation

Separation of the polyphosphate species was implemented after a number of Phase I tests were already completed. Repeated analysis over time of phosphate concentrations (by IC) had revealed that conversion to phosphate was much slower than initially anticipated. Detection limits for the IC were 0.02 $\mu\text{g/mL}$ for $\text{PO}_4^{3-}\text{-P}$, 0.01 $\mu\text{g/mL}$ $\text{PO}_3^{2-}\text{-P}$ and 0.03 to 0.05 $\mu\text{g/mL}$ $\text{PO}_2^{2-}\text{-P}$.

Separation of polyphosphate species on neutralized and unneutralized WP-F stock solutions in a range of water types is shown in Tables 17 and 18. These solutions ranged in age from 23 to 93 days since preparation. Earliest HPLC separations (Table 17) employed Na_3EDTA -containing eluents in an effort to maximize separation of peaks. This eluent caused the 3P and 4P chains to coelute, however. Later HPLC separations (Table 18) used Na_4EDTA -containing eluents, which successfully separated 3P and 4P chains at only a small decrease in retention time intervals of the other polyphosphates. The neutralized stock solutions show a striking change in hydrolysis products. The differences for very hard water were not as dramatic, presumably because of the higher initial pH following partial neutralization of the acidic smoke by the constituents of the water. The larger polyphosphate chains have disappeared in the neutralized stocks, while the level of cyclic trimetaphosphate is greatly enhanced, and small amounts of tetra- and hexametaphosphate are suspected. The observed levels

TABLE 17. DISTRIBUTION OF PHOSPHORUS SPECIES IN NEUTRALIZED AND NON-NEUTRALIZED WP-F SOLUTIONS FROM SMOKE CONTACTS AFTER AGING

	Well Water at 39 days		Very Hard Water at 40 days		Hard Water at 23 days	
pH (initial)	2.8	7.7	4.9	8.1	2.6	8.0
P Species ^a	- - - - - %P _{tot} - - - - -					
1P	22.1	22.8	22.8	30.3	19.6	21.7
2P	23.0	20.0	19.4	16.0	16.3	12.3
3 + 4P	26.8	40.9 ^b	36.1	38.6	21.0	31.9
5P	5.5	<DL	1.8	<DL	7.5	5.9
6P	4.1	0.6	0.4	<DL	6.2	1.6
7P	3.8	0.4	0.1	<DL	5.2	0.9
8P	2.8	0.1	0.2	<DL	4.8	<DL
9P + 6mP	2.6	0.7	0.9	0.5	4.5	1.0
10P + 4mP	1.9	0.5	0.6	0.6	3.3	0.7
11P	1.1	<DL	<DL	<DL	2.6	<DL
12P	0.7	<DL	<DL	<DL	1.9	<DL
13P + 3mP	3.8	14.1	17.8	14.2	3.3	24.2
>14P	1.7	<DL	<DL	<DL	2.8	<DL

^a $nP \equiv (P_nO_{3n+1})^{-(n+2)}$; $nmP \equiv (P_nO_{3n})^{-n}$

^b Below detection limit (0.1%).

of phosphate with age gave little indication of the change in hydrolysis in neutralized solutions with time. Apparently a rate-determining step in overall hydrolysis could involve the 3P and 3mP species (Table 18).

Speciations of RP-BR combustion products was conducted with the stock solution generated in the first trial burn on this project and on a smoke-dosed deionized water. Apparently, the salt content (Ca, etc.) of the well water has some stabilizing effect on the polyphosphate species, such that even after 6 months there were measurable levels of chains up to 12P (Table 19). Early breakdown of long-chain polyphosphates was demonstrated by speciation of deionized water dosed sample over a 9-day period. Phosphate formation in distilled water dosed with WP-F smoke (Burn WP-7)

TABLE 18. DISTRIBUTION OF PHOSPHORUS SPECIES IN HARD WATER SOLUTIONS USING Na₄ EDTA Eluents

pH (initial)	48 days		93 days
	2.6	8.0 ^a	2.6
P Species ^b	%P _{TOT}		
1 P	21.0	22.6	25.8
2 P	14.2	12.0	19.2
3 P	12.5	21.4	12.3
4 P	10.2	10.2	9.1
5 P	7.0	3.1	6.0
6 P	5.3	0.4	4.3
7 P	4.7	0.1	3.6
8 P	4.3	0.06	3.0
9 P	3.5	0.04	2.8
10 + 6 mP	3.5	1.2	2.8
11 P	2.1	<DL ^c	1.7
12 + 4 mP	2.0	0.6	1.6
13 P	1.4	<DL	1.1
14 P	1.4	<DL	1.3
15 + 3 mP	2.8	28.3	3.9
16 P	1.3	<DL	0.4
17 P	0.9	<DL	0.5
18 P	0.7	<DL	0.2
19 P	0.5	<DL	0.2
20 P	0.3	<DL	0.1
21 P	0.1	<DL	<DL
22 P	0.1	<DL	<DL
23 P	0.1	<DL	<DL

^a Neutralized at day 0.

^b $nP \equiv (P_nO_{3n+1})^{-(n+2)}$; $nmP \equiv (P_nO_{3n})^{-n}$

^c Below detection limit (0.1%).

TABLE 19. PHOSPHORUS DISTRIBUTION WITH TIME IN RP-BR SMOKE
CONTACTED WATERS

P Species	Age (days) at HPLC Analysis			
	Bubbled Well water	De-ionized water ^a Static Deposition		
	180	0.7	1.7	9.0
	----- %P _{tot} -----			
1P	48.5	23.4	25.8	30.3
2P	18.1	9.6	9.2	11.8
3P	22.3 ^b	6.8	7.6	10.5
4P		6.8	9.5	13.4
5P	1.9	7.3	9.6	9.8
6P	0.9	6.9	8.2	5.1
7P	0.9	6.2	6.3	2.6
8P	0.6	5.7	4.9	0.9
9P	<0.5	4.8	3.6	0.5
10P + 6mP	~1.1 max	4.5	2.9	0.5
11P	<0.5	3.5	1.8	b
12P + 4mP	~0.8 max	3.0	1.6	0.7
13P	<DL ^d	3.1	c	c
14P	<DL	1.6	0.8	c
15P + 3mP	4.8	2.0	7.9	13.9
16P	<DL	1.4	c	<DL
17P	<DL	0.9	<0.2	<DL
18P	<DL	1.1	<DL	<DL
19P	<DL	0.5	<DL	<DL
20P	<DL	0.4	<DL	<DL
21P	<DL	0.3	<DL	<DL
22P	<DL	0.2	<DL	<DL
23P	<DL	0.15	<DL	<DL
24P	<DL	0.15	<DL	<DL

^a Data from Van Voris et al. 1984.

^b 4P species coeluted with 3P when eluent contained Na₃EDTA; total for both species was 22.3%.

^c Region masked by mP peaks; estimated to be ≤0.2%.

^d Below detection limit.

showed a similar rate of hydrolysis over a 1-month period to that of a well water dosed during the same test.

Phase I and Phase II Stock Generations

Tables 20 and 21 summarize the phosphorus data taken for the stock solutions used in Phase I and Phase II testing, respectively. Changes with dilution in percentage of P as phosphate or phosphite were minor when analyzed fresh and precipitation had not occurred. The phosphate levels generated in Phase I testing (Table 20) did not follow any trend versus water hardness or smoking agent, but were influenced by run-to-run differences that likely occurred during smoke generation. Phosphate levels during Phase II tests (Table 21) were generally higher than during Phase I tests, at least partly due to the change in smoke dosing configuration and settling time in the 760-L Fusion carboys. It is not known whether an increase in trimetaphosphate also occurred, or whether the breakdown of polyphosphates in these unneutralized solutions proceeded primarily to the shorter chain (2P-6P) linear polyphosphates.

The higher level of phosphite generated during Phase I RP-BR versus WP-F smoke-dosed waters was generally repeated in Phase II tests, though the difference was not as significant and the spread in sample values was greater. Apparently there was enough of a difference in the combustion of WP-F to keep the phosphite production minimized.

3.2.2 Toxicity

This section covers the toxic effects of P-based obscurants. Phase I testing covered preliminary tests with D. magna on the interactions among water quality, aging of exposure solutions and toxicity. The ability of phosphorus smoke to acidify water was related to the buffering capacity of the water. Precipitation of P and alkaline earth elements was more prevalent in the more buffered waters. Phase II consisted of definitive toxicity tests with four species of fish, four species of invertebrates and a green algae.

Phase I Tests

During Phase I of the project, several tests were performed to evaluate the mode of toxicity of these obscurants. Daphnia magna was the only species tested during these tests. The battery of tests performed included 96-hr toxicity screening tests, tests on the effects of buffering capacity of the diluent on toxicity, and the effects of aging of exposure solutions on toxicity.

Initial Tests. The initial tests performed in well water with D. magna were conducted for 96-hr. A bimodal response was noted (Table 22) indicating that within the initial 48 hr of exposure, the principal toxic effect was related to acidification resulting from hydrolysis of the

TABLE 20. RP-BR AND WP-F STOCK SOLUTIONS USED WITH DAPHNIA MAGNA
TOXICITY TESTS DURING PHASE I

Test ID	Water Type	pH	Total P (mg/L)	% as $\text{HPO}_4^{2-}\text{-P}$	% as $\text{PO}_4^{3-}\text{-P}$
-----RP-BR Exposures-----					
RP-1 ^a	331 Well	3.0	116	2.50	16.9
RP-2	331 Well	6.4	31.0	0.10	10.0
RP-3	331 Well	3.4	99.9	1.06	6.6
RP-3	331 Well	7.0	33.7	1.04	6.9
RP-3	331 Well	7.2	24.2	1.03	7.1
RP-3	331 Well	7.3	16.6	1.02	6.8
RP-4	331 Well	3.2	101	0.41	13.5
RP-5	331 Well	3.2	93.1	0.59	14.4
RP-5	331 Well, Neutralized	8.0	93.1	0.59	14.4
RP-5	Very Hard	6.8	96.3	0.57	12.7
RP-5	Very Hard, Neutralized	8.0	95.2	0.52	11.8
RP-9	Soft	2.9	69.9	0.36	12.7
RP-9	Soft, Neutralized	7.2	71.3	0.33	12.3
RP-15	Hard	5.5	65.4	0.56	7.3
RP-15	Hard, Neutralized	8.1	64.8	0.59	7.4
RP-16	Hard	4.7	69.1	0.45	7.7
RP-16	Hard, Neutralized	8.3	68.7	0.45	7.7
-----WP-F Exposures-----					
WP-2	331 Well	2.2	368	0.09	8.5
WP-3	331 Well	2.2	380	0.15	11.1
WP-4	331 Well	5.1	67.3	0.12	10.6
WP-4	331 Well, Neutralized	7.6	63.8	0.12	10.7
WP-4	Soft	2.8	66.4	0.11	11.4
WP-4	Soft, Neutralized	7.4	68.1	0.12	11.2
WP-5	331 Well	2.9	101	0.07	14.9
WP-6	331 Well	2.8	132	0.10	14.7
WP-6	331 Well, Neutralized	8.1	132	0.12	14.6
WP-6	Very Hard	4.9	132	0.11	18.7
WP-6	Very Hard, Neutralized	7.9	132	0.11	18.0
WP-7	331 Well	2.8	120	0.10	13.7
WP-7	331 Well, Neutralized	7.7	120	0.10	13.7
WP-7	Very Hard	4.9	125	0.08	13.2
WP-7	Very Hard, Neutralized	8.1	96.4 ^b	0.10	19.3
WP-7	Distilled Water ^c	2.6	74.6	0.10	16.5
WP-8	Hard	2.6	163	0.12	14.5
WP-8	Hard, Neutralized	8.0	168	0.10	14.5
WP-10	Hard	3.1	88.6	0.08	14.7
WP-10	Hard, Neutralized	8.1	89.4	0.08	14.3

^a Sampled before washdown of container sides. This is the only bubbled

smoke-dosed water sample.

^b Precipitation occurring even in stock solution in less than 8 hr.

^c Not exposed at same time as other waters, so change in Total P is not surprising.

TABLE 21. RP-BR AND WP-F STOCK SOLUTIONS USED DURING PHASE II
TESTS WITH COLUMBIA RIVER WATER

Test ID	Species	pH	Total P (mg/L)	% as $\text{HPO}_4^{2-}\text{-P}$	% as $\text{PO}_4^{3-}\text{-P}$
RP-9	<u>D. magna</u>	4.4	34.4	0.06	6.8
RP-13	<u>Hexagenia</u> sp.	2.9	148	0.67	15.1
RP-12	<u>H. azteca</u>	3.2	60	0.21	12.3
RP-12	<u>P. dissimilis</u>	3.2	60	0.21	12.3
RP-10	<u>P. promelas</u>	2.4	282	0.22	22.9
RP-16	<u>S. gairdneri</u>	2.4	198	0.43	19.3
RP-11	<u>T. punctatus</u>	2.1	514	0.13	40.3
RP-14	<u>T. punctatus</u>	2.3	198	0.63	15.3
RP-12	<u>L. macrochirus</u>	2.1	537	0.12	34.4
RP-7	<u>S. capricornutum</u>	7.9 ^a	75	0.19	10.3
RP-8	<u>S. capricornutum</u>	2.7	97	0.16	11.3
RP-14	<u>S. capricornutum</u>	2.7	103	0.69	8.1
WP-16 ^b	<u>D. magna</u>	2.5	145	0.11	17.8
WP-15	<u>Hexagenia</u> sp.	2.2	410	0.11	20.2
WP-14	<u>H. azteca</u>	2.1	430	0.13	37.9
WP-14	<u>P. dissimilis</u>	2.1	430	0.13	37.9
WP-14	<u>P. promelas</u>	2.1	447	0.05	22.0
WP-15	<u>S. gairdneri</u>	2.2	410	0.11	20.2
WP-13	<u>T. punctatus</u>	2.1	588	0.14	30.1
WP-16	<u>L. macrochirus</u>	2.0	548	0.08	39.0
WP-11	<u>S. capricornutum</u>	2.6	125	0.08	13.9
WP-14	<u>S. capricornutum</u>	2.7	42.8	0.09	26.0

^a EPA media with high added alkalinity (390 mg/L) compared to normal media (16 mg/L).

^b Stock solution had been frozen for 3 days before exposure of organisms.

TABLE 22. BIMODAL RESPONSE OF D. MAGNA OVER 96 HR, BURN RP-5

Dilution	Total P (mg/L) ^a	Mortality			
		24 hr	48 hr	72 hr	96 hr
Control		0	0	0	0
10%	(9.3)	0	0	0	0
30%	(27.9)	0	0	0	29.2
40%	(37.2)	0	0	16.7	79.2
50%	(46.6)	0	0	4.2	83.3
70%	(65.2)	33.3	91.7	100	100
100%	93.1	100	100	100	100

^a Values in parenthesis are nominal concentrations.

P combustion products in water. At 96 hr, non-dose-dependent mortality was observed in the 30% to 50% dilutions. Coincident with the mortality was the formation of precipitated material in the exposure beakers and in the gastrointestinal (GI) tract of the exposed daphnids. This generalized mortality pattern was observed in other Phase I tests and may be related to physical effects (accumulation of precipitated material by the organisms), chemical toxicity or a combination of the two factors. This bimodal response was unique to D. magna and was not observed in any other species tested during Phase II of the project.

Aging Tests. It was not known how the concentration of phosphorus combustion products in well water would effect the rate of hydrolysis over the duration of the aging tests. Hydrolysis rates may also be influenced by pH. By initially preparing sufficient amounts of exposure solutions for the 26- to 28-day period of testing, the effect that acidification would have on stabilization of the combustion products in water was minimized. The 40% solutions that were prepared at days 5 and 26 served as a check on the toxicity of the more acidified stock solutions (pH ~3.0) over time. In the test with WP-F, toxicity observed at 48 hr at the three test periods was principally attributed to pH (Table 23). That the mortality observed at the 20% to 50% solutions for the Day 5 test exceeded the observed mortality for the same solutions at Day 0 may be explained by random variability in the overall sensitivity of the test populations of daphnids. This explanation is supported by the observation that a higher level of mortality was observed at 96 hr in the 20 to 50% solutions for the Day 0 tests than in the Day 5 test. This implies that the group of daphnids tested at day 5 were more sensitive over all than the Day 0 population. This point is further corroborated by the observation that mortality in the control treatment for the Day 5 test at 96 hr was five times as high as the Day 0 test. The

TABLE 23. TOXICITY (% MORTALITY, N = 24) OF WP-F SMOKE-DOSED WATER TO DAPHNIA MAGNA AS A FUNCTION OF THE AGE OF EXPOSURE SOLUTION (BURN WP-5)^a

Treatment	Initial pH ^b	24 hr	48 hr	72 hr	96 hr
-----Day 0-----					
70%	4.61	100.0%	100.0%	100.0%	100.0%
50%	5.89	4.2%	8.3%	50.0%	95.8%
40%	6.28	0	4.2%	79.2%	100.0%
30%	6.56	0	0	79.2%	100.0%
20%	6.61	4.2%	4.2%	79.2%	100.0%
10%	7.18	0	0	0	29.2%
Control	7.95	0	0	0	4.2%
-----Day 5-----					
70%	4.84	100.0%	100.0%	100.0%	100.0%
50%	6.83	12.5%	45.8%	70.8%	83.5%
40%	7.62	8.3%	41.7%	50.0%	54.2%
30%	7.96	4.2%	37.5%	54.2%	62.5%
20%	8.21	12.5%	33.3%	37.5%	50.0%
10%	8.43	4.2%	4.2%	4.2%	12.5%
Control	8.59	0	4.2%	8.3%	25.0%
40% from Day 0, filtered	7.73	12.5%	29.2%	37.5%	37.5%
40% from 5 day aged stock solution	6.45	8.3%	50.0%	87.5%	100.0%
-----Day 28-----					
70%	4.67	100.0%	100.0%	100.0%	100.0%
50%	6.94	0	4.2%	4.2%	4.2%
40%	7.51	0	0	0	0
30%	7.91	0	0	0	0
20%	8.21	0	0	0	4.2%
10%	8.39	0	0	0	0
Control	8.50	0	0	0	0
40% from 28 day aged stock solution	7.49	0	0	20.8%	50.0%

^a See Table 14 for comparison of phosphate and total phosphorous concentrations as a function of solution age.

^b Measured in the exposure beakers.

bimodal responses noted at 96 hr revealed an effect of aging on the exposure solutions. At 0 and 5 days of aging, there was no apparent difference in response. At 28 days, however, there was a clear and distinctive decrease in toxicity reflected in the 96-hr data.

The same generalized conclusions can be drawn from the aging tests with RP-BR (Table 24). In this series of tests, the observed mortality at 96 hr in the 20 to 50% dilutions was lower than that observed in the WP-F series at 0 and 5 days aging.

Auxiliary exposures were set up during these tests. Exposure solutions from the 40% dilutions from the Day 0 test were filtered (0.22 μ m) and retested during the Day 5 tests for WP-F and RB-BR obscitants. At the second and third test in each series, a "fresh" 40% dilution was prepared from the original day 0 stock solution. The following interpretation is based on the observed toxicity at 72 and 96 hr. Toxicity of freshly prepared RP-BR 40% exposure solutions showed a slight increase over 26 days. The Day 5 auxiliary test beakers were loaded with first daphnid instars after the rest of the test had been set up. This may have introduced some bias into the results, i.e., the apparent increase in toxicity noted in the fresh 40% solution compared to the aged 40% solution may be due to the fact that the daphnids remaining after loading the exposure beakers for the Day 5 test may have been weaker organisms. Nonetheless, the difference between the fresh and the aged RP-BR 40% solutions at 26 days is quite apparent and not biased by loading sequence. In both tests, the filtered 40% solutions at 5 days were less toxic than the fresh 40% solutions.

Neutralization Tests. Neutralization tests with D. magna were performed in dilution water with four distinct levels of buffering capacity; well water and three levels of reconstituted water. The buffering capacity of these waters ranged from 50 mg/L to 225 mg/L carbonate alkalinity. Because the primary mode of toxicity was acidification and the method of dosing water generally resulted in stock solutions with a total P concentration of 70 to 120 mg/L, the pattern of toxic responses as a function of dilution showed an increased sensitivity of first instar D. magna in reconstituted soft water compared to well water and the more buffered reconstituted waters. A complete accounting of mortality records is located in Appendix C.

For comparative purposes, only the exposure concentrations showing the highest observed mortality (%) and the next lowest exposure solution for each type of water are compared in Table 25. For example, the 30% solution of reconstituted soft water dosed with WP-F smoke (19.9 mg/L total P, pH 4.71) was lethal to 100% of the exposed organisms. The next lower concentration tested usually had 0% mortality. Overall, Table 25 gives an indication of how the buffering capacity of the receiving water affects toxicity. There are toxic components present in smoke-dosed water as indicated by the mortality observed in the neutralized and non-neutralized exposure solutions that were tested in all four types of dilution water.

TABLE 24. TOXICITY (% MORTALITY, n = 24) OF RP-BR SMOKE TO DAPHNIA
MAGNA AS A FUNCTION OF AGE OF THE EXPOSURE SOLUTIONS

Treatment	Initial pH ^a	24 hr	48 hr	72 hr	96 hr
-----Day 0-----					
70%	5.10	100.0%	100.0%	100.0%	100.0%
50%	6.25	0	0	12.5%	25.0%
40%	6.50	0	0	16.7%	25.0%
30%	6.75	0	0	8.3%	12.5%
20%	7.01	0	0	4.2%	4.2%
10%	7.25	0	0	0	0
5%	7.55	0	0	0	0
Control	7.90	0	0	0	0
-----Day 5-----					
70%	4.93	69.6%	100.0%	100.0%	100.0%
50%	7.03	0	0	0	2.4%
40%	7.52	0	0	4.2%	8.3%
30%	7.95	0	4.2%	4.2%	8.3%
20%	8.14	0	0	0	0
10%	8.29	0	0	0	0
5%	8.40	0	0	0	0
Control	8.42	0	0	0	0
Day 0 "filtered"					
40% dilution	7.48	0	0	4.2%	33.3%
40% dilution prepared from 5 day stock solution	6.61	0	0	20.8%	75.0%
-----Day 26-----					
70%	5.06	58.3%	100.0%	100.0%	100.0%
50%	7.05	0	0	0	0
40%	7.49	0	0	4.2%	4.2%
30%	7.98	0	0	0	0
20%	8.19	0	0	0	0
10%	8.34	0	0	0	0
5%	8.43	0	0	0	0
Control	8.31	0	0	0	0
40% prepared from 25 day stock solution	6.57	0	0	70.8%	70.8%

^a Measured in the exposure beakers.

TABLE 25. COMPARISON OF MORTALITY OF DAPHNIA MAGNA IN NEUTRALIZATION TESTS WITH FOUR TYPES OF DILUTION WATERS^a

Condition	RP-BR		WP-F	
	Neutralized	Non-Neutralized	Neutralized	Non-Neutralized
-----Soft Water-----				
Mortality (%)	100	100	88	100
pH	7.25	2.92	7.69	4.71
Total P (mg/L)	71.3	69.9	68.1	19.9
Mortality (%)	0	0	0	0
pH	7.19	5.73	7.48	6.71
Total P (mg/L)	14.3	14.0	47.7	6.63
-----Hard Water-----				
Mortality (%)	0	100	40	100
pH	7.96	4.70	7.97	3.91
Total P (mg/L)	69.1	69.1	89.4	53.2
Mortality (%)	--	0	5	10
pH	--	6.29	7.98	5.99
Total P (mg/L)	--	48.4	71.7	35.4
-----Well Water-----				
Mortality (%)	0 ^b	91.7 ^b	4.2	100
pH	7.43	5.47	7.78	5.38
Total P (mg/L)	93.1	65.2	66.4	66.4
Mortality (%)	--	0	0	0
pH	--	6.82	7.81	6.67
Total P (mg/L)	--	46.6	46.5	46.8
-----Very Hard Water-----				
Mortality (%)	0	0	30	100
pH	7.80	7.13	8.08	4.99
Total P (mg/L)	95.2	96.3	125	125 ^c
Mortality (%)	--	--	10	0
pH	--	--	8.31	6.55
Total P (mg/L)	--	--	87.6	87.5

^a Only the treatments with the highest observed mortality are compared with the next lowest concentration. Mortality measurements were taken at 48 hr. (See Appendix C for complete data sets.)

^b Test 2 (Burn RP-16).

^c Nominal concentration at time test was started, analysis of sample after 8 hr of agings was 96.4 mg/L, Burn WP-7.

However, the toxicity of neutralized and non-neutralized RP-BR solutions in soft water appeared to be identical in terms of total P. The toxic component(s) of the stock solutions were not identified, but it is apparent that a considerable amount of smoke is necessary to induce non-pH related mortality in a body of water irrespective of the buffering capacity of that water. Median lethal concentration (LC50) values were not calculated for these data sets because they generally lacked treatments with partial mortality. Mortality between 48 and 96 hr represents interactions with precipitated material.

Phase II Tests

It is readily apparent from the Phase I tests with D. magna that the primary mode of toxicity of phosphorus based smokes was acidification. The dilution water used for Phase II testing was Columbia River water. The carbonate buffering capacity of river water seasonally ranges from 55 to 65 mg/L as CaCO_3 . The results of acute toxicity tests for fish and invertebrates conducted during Phase II are reported as a function of pH and total phosphorus. The response of S. capricornutum to smoke-dosed algal media was evaluated from a toxicological perspective and from the ability of phosphorus combustion products to stimulate algal growth.

S. capricornutum Toxicity Tests. The analysis of variance (ANOVA) of cell count data indicated significant differences for treatment means, time and interactions in the algal toxicity test with RP-BR (Table 26). The only significant differences in treatment means were observed at 96 hr. Cell count data for all sample periods are summarized in Appendix A. The 5% exposure solution was significantly different ($p \sim 0.05$) compared with all other treatments at 96 hr (Tables 27 and 28). The overall trends in this test suggest stimulation of algal growth at 1 and 4.6 mg/L total P with the

TABLE 26. ANALYSIS OF VARIANCE (ANOVA) OF MEAN CELL COUNT DATA FROM THE ALGAL TOXICITY TEST WITH RP-BR

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Prob.
Mean	2228910.87184	1	2228910.87184	58.05	0.0000
Cell Counts	6463387.15352	7	923341.02193	24.05	0.0000
Error	614382.05409	16	38398.87838		
Time	4886365.97658	4	1221591.49414	38.83	0.0000
Interaction	21736491.33644	28	776303.26202	24.68	0.0000
Error	2013335.02675	64	31458.35979		

TABLE 27. F-VALUES FOR COMPARISONS BETWEEN ALL POSSIBLE PAIRS OF MEANS AT 0, 24, 48, 72, AND 96 HOURS FOR RP-BR TOXICITY TEST WITH S. CAPRICORNUTUM

Treatment Contrast	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
100% - 10%	0.00	0.00	0.00	0.00	0.00
100% - 9%	0.00	0.00	0.00	0.00	0.00
100% - 7%	0.00	0.00	0.00	0.00	0.05
100% - 5%	0.00	0.04	0.92	0.35	434.44 ^a
100% - 1%	0.00	0.03	0.26	0.04	11.63
100% - Control	0.00	0.03	0.44	0.36	1.09
100% - Neut. Stock	0.01	0.01	0.01	0.01	0.02
10% - 9%	0.00	0.00	0.00	0.00	0.00
10% - 7%	0.00	0.00	0.00	0.00	0.08
10% - 5%	0.00	0.03	0.87	0.31	437.20 ^a
10% - 1%	0.00	0.02	0.23	0.03	12.08
10% - Control	0.00	0.02	0.40	0.33	1.23
10% - Neut. Stock	0.00	0.01	0.00	0.00	0.04
9% - 7%	0.00	0.00	0.00	0.00	0.06
9% - 5%	0.00	0.03	0.89	0.32	435.82 ^a
9% - 1%	0.00	0.02	0.24	0.03	11.86
9% - Control	0.00	0.02	0.42	0.33	1.16
9% - Neut. Stock	0.00	0.01	0.00	0.00	0.02
7% - 5%	0.00	0.02	0.87	0.36	425.43 ^a
7% - 1%	0.00	0.01	0.23	0.04	10.20
7% - Control	0.00	0.01	0.40	0.37	0.68
7% - Neut. Stock	0.00	0.00	0.00	0.01	0.01
5% - 1%	0.00	0.00	0.21	0.15	303.91 ^a
5% - Control	0.00	0.00	0.09	0.00	392.04 ^a
5% - Neut. Stock	0.00	0.01	0.78	0.26	429.34 ^a
1% - Control	0.00	0.00	0.02	0.16	5.60
1% - Neut. Stock	0.00	0.00	0.18	0.01	10.81
Control - Neut. Stock	0.00	0.00	0.34	0.27	0.85

^a Significant of $p < .05$ using Scheffé's test with error rate adjustment for simultaneous comparisons.

toxic effects of acidification inhibiting growth at pH levels ~5.0. Growth in the excessively buffered stock solution from burn RP-7 was inhibited to the same degree as the acidified stock solution suggesting that unidentified components of the stock solution were toxic independent of pH.

TABLE 28. RESPONSE OF SELENASTRUM CAPRICORNUTUM TO RP-BR SMOKE-DOSED WATER
(96-HR DATA)

Treatment (%)	Total P (mg/L)	pH	Mean (\pm 1.0 S.D.) Cell Counts in L x 10 ⁶
100	97	2.72 ^a	0.03 \pm 0.02
10	9.3	5.03 ^a	0.02 \pm 0.00
9	8.6	4.95 ^a	0.02 \pm 0.02
7	6.1	5.70 ^a	0.06 \pm 0.07
5	4.6	7.04 ^a	3.36 \pm 0.80 ^d
1	1.0	7.76 ^a	0.57 \pm 0.80
Control ^b	(<DL) ^c	7.8 ^a	0.20 \pm 0.12
100 ^b	75	7.9	0.05 \pm 0.01

^a Measured at end of test (RP-8).

^b Neutralized stock solution with 360 mg/L total alkalinity (RP-7).

^c Below detection limit.

^d Significant at $p \leq 0.05$

The response of S. capricornutum to WP-F smoke-dosed algae media was similar to that of RP-BR except that the responses within treatments were more variable. The high level of variability may be related to the size of the inoculum at the start of the test. The ANOVA showed no significant differences in treatment means, time or interactions (Table 29) and no additional statistical tests were done. Trends in the 96-hr data (Table 30) suggest that inhibition of growth was occurring at pH levels of ~6.0; however, the high level of variability prevents any conclusive statements regarding growth stimulation.

S. capricornutum Growth Stimulation Tests. The analysis of RP-BR and WP-F algal stimulation tests were based on mean cell counts determined with a Coulter counter. Chlorophyll analyses were also performed, but the data were more variable than the Coulter counter data and were not analyzed (Appendix A). It has been summarized in the tables for comparison with the coulter counter data only.

Algal stimulation tests were performed with neutralized and non-neutralized solutions of smoke-dosed algal media with RP-BR and WP-F. Cell count data for all sample periods are summarized in Appendix A. The repeated measures ANOVA for the RP-BR test (Table 31) indicated significant main effects, i.e., pH (neutralized vs. non-neutralized exposure solutions), total phosphorus concentrations and time, and all possible interactions.

TABLE 29. ANALYSIS OF VARIANCE (ANOVA) OF MEAN CELL COUNT DATA FROM THE ALGAL TOXICITY TEST WITH WP-F

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Prob.
Mean	576622.29515	1	576622.29515	11.25	0.0047
Cell Counts	337156.74826	6	56192.79138	1.10	0.4113
Error	717357.50717	14	51239.82194		
Time	1004987.04523	4	251246.76131	7.90	0.0000
Interaction	849333.35094	24	35388.88962	1.11	0.3604
Error	1780065.71975	56	31786.88785		

TABLE 30. RESPONSE OF SELENASTRUM CAPRICORNUTUM TO WP-F SMOKE-DOSED WATER (96-HR DATA)

Treatment (%)	Total P	pH	Mean (± 1.0 S.D.) Cell Counts ^a
100	12.5	2.55	0.02 \pm 0.02
6	7.6	6.06	0.05 \pm 0.04
3	3.6	6.26	0.66 \pm 0.55
1	1.8	6.77	0.43 \pm 0.48
0.6	0.7	7.02	0.26 \pm 0.42
Control	<DL ^b	7.44	0.42 \pm 0.68
Neut. Stock	12.5	7.32	0.02 \pm 0.00

^a All counts $\times 10^6$ /mL

^b Below detection limit.

TABLE 31. ANALYSIS OF VARIANCE ANOVA OF ALGAL STIMULATION TESTS WITH RP-BR (BASED ON 96-HR MEAN CELL COUNTS)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Prob.
Mean	16986771.88991	1	16986771.88991	1444.76	0.0000
Phosphorus Level	1320084.16965	4	330021.04241	28.07	0.0000
Neutralization	765093.50002	1	765093.50002	65.07	0.0000
Phosphorus vs. Neutralization/ Non-neutralization	1201015.29635	4	300253.82409	25.54	0.0000
Error	329209.04702	28	11757.46596		
Interactions					
Time	24018794.18772	4	6004698.54693	921.76	0.0000
Time vs. Phosphorus	3333319.42322	16	208332.46395	31.98	0.0000
Time vs. Neutralization	1269295.64087	4	317323.91022	48.71	0.0000
Time x Phosphorus x Neutralization	2297734.17342	16	143608.38584	22.04	0.0000
Error	729613.07148	112	6514.40242		

There were no significant differences in mean cell counts until 96 hr (Table 32). At that time, the response of the neutralized treatments (pH 7.15 to 7.25) were significantly different ($\alpha = 0.05$ with Scheffé's correction) than the acidified 5.0% (pH 4.8) and the 3.3% (pH 5.5) solutions (Tables 32 and 33). The difference in growth indicated inhibition due to acidification of the medium further suggesting that acidification was the primary mode of toxicity. A trend toward higher levels of growth as a function of increasing P concentration was noted. There were no differences at any time in response within the neutralized treatments. In the non-neutralized treatments, however, the 96-hr mean cell count in the 4.98% solution was significantly different than the three more diluted treatments and the 3.27% solution was significantly different than the 2.77% and the 1.81% solutions. Moreover, mean cell counts in the PO_4^{3-} deficient media were significantly lower (using Scheffé's corrections) than all four neutralized treatments and the 2.77% and 1.81% non-neutralized treatments. Mean cell counts in the EPA media control treatment were twice that of the PO_4^{3-} deficient control.

TABLE 32. F-VALUES FOR COMPARISONS BETWEEN ALL POSSIBLE PAIRS OF MEANS AT 0, 24, 48, 72, AND 96 HOURS FOR RP-BR ALGAL STIMULATION TESTS

Treatment Contrast	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
4.98% Non-Neut. - 4.98% Neut	0.04	0.20	3.32	33.19	422.33 ^a
3.27% Non-Neut. - 3.27% Neut	0.00	0.00	1.60	12.85	173.31 ^a
2.77% Non-Neut. - 1.81% Neut	0.01	0.19	0.10	0.01	0.11
1.81% Non-Neut. - 2.77% Neut	0.01	0.02	0.04	0.40	4.14
P0 ₄ ⁻ Def. Control - 4.98% Non-Neut	0.01	0.06	1.66	24.05	52.93
P0 ₄ ⁻ Def. Control - 3.29% Non-Neut	0.01	0.00	0.00	1.07	9.05
P0 ₄ ⁻ Def. Control - 2.77% Non-Neut	0.00	0.06	0.97	2.43	159.40 ^a
P0 ₄ ⁻ Def. Control - 1.81% Non-Neut	0.00	0.00	0.17	2.99	273.67 ^a
4.98% Non-Neut. - 3.27% Non-Neut	0.00	0.10	1.76	14.97	105.74 ^a
4.98% Non-Neut. - 2.77% Non-Neut	0.03	0.00	5.18	41.75	396.03 ^a
4.98% Non-Neut. - 1.81% Non-Neut	0.02	0.06	2.90	44.00	567.31 ^a
3.27% Non-Neut. - 2.77% Non-Neut	0.02	0.09	0.90	6.72	92.50
3.27% Non-Neut. - 1.81% Non-Neut	0.01	0.00	0.14	7.64	183.20 ^a
2.77% Non-Neut. - 1.81% Non-Neut	0.00	0.05	0.33	0.03	15.35
P0 ₄ ⁻ Def. Control - 4.98% Neut	0.09	0.06	0.59	3.09	213.47 ^a
P0 ₄ ⁻ Def. Control - 3.27% Neut	0.01	0.01	1.70	6.51	261.55 ^a
P0 ₄ ⁻ Def. Control - 1.81% Neut	0.00	0.04	0.45	2.11	167.85 ^a
P0 ₄ ⁻ Def. Control - 2.77% Neut	0.01	0.02	0.38	5.59	210.47 ^a
4.97% Neut. - 3.27% Neut	0.05	0.03	0.09	0.11	1.98
4.97% Neut. - 1.81% Neut	0.07	0.01	0.05	0.33	16.26
4.97% Neut. - 2.77% Neut	0.05	0.01	0.07	0.03	7.65
3.27% Neut. - 1.81% Neut	0.00	0.01	0.40	1.21	10.35
3.27% Neut. - 2.77% Neut	0.00	0.00	0.47	0.03	2.77
1.81% Neut. - 2.77% Neut	0.00	0.00	0.00	0.83	2.41

^a Significant at p<.05 using Scheffe's test with error rate adjustment for simultaneous comparisons.

TABLE 33. RESPONSE OF SELENASTRUM CAPRICORNUTUM IN ALGAL STIMULATION TESTS WITH RP-BR SMOKE-DOSED PO_4^{3-} DEFICIENT MEDIA (96-HR DATA)

Treatment (% Stock Solution)	Nominal Total P (mg/L)	PO_4^{3-} (mg/L)	Initial pH	Mean (± 1.0 S.D.) Cell Counts ($\times 10^6$ /mL)	Ratio Cell Counts to PO_4^{3-} Deficient Controls	Mean (± 1.0 S.D.) Chlorophyll A (mg/L)	Ratio Chlorophyll to PO_4^{3-} Deficient Controls
-----Non-neutralized-----							
4.98%	5.13	1.41	4.95	0.01 ± 0.00	0.02	0.70 ± 0.20	0.02
3.27%	3.37	1.13	5.2	0.65 ± 0.25	1.41	54.88 ± 39.68	1.72
2.77%	2.85	0.86	6.08	1.24 ± 0.15	2.70	189.95 ± 33.47	5.94
1.81%	1.91	0.61	6.48	1.48 ± 0.13	3.22	123.12 ± 35.81	3.85
-----Neutralized-----							
4.98%	5.13	1.38	7.20	1.59 ± 0.16	3.46	187.31 ± 128.20	5.86
3.27%	3.37	0.92	7.15	1.45 ± 0.08	3.15	281.01 ± 97.05	8.79
2.77%	2.85	0.83	7.24	1.26 ± 0.20	2.74	118.40 ± 50.92	3.70
1.81%	1.91	0.64	7.25	1.35 ± 0.09	2.93	182.51 ± 49.80	5.71
-----Controls-----							
PO_4^{3-} Deficient Media	0.15	0.25	7.46	0.46 ± 0.17	--	31.97 ± 38.31	--
Complete Media	--	0.69	7.50	0.83 ± 0.21	1.81	32.32 ± 27.38	1.01

The responses observed in the WP-F algal stimulation test were generally similar to those in the RP-BR test. All main and interaction effects were significant ($\alpha = 0.5$, Table 34). At 72 and 96 hr, the individual means test showed that the non-neutralized 10% treatment was significantly different ($\alpha = 0.05$ with Scheffé's correction) than the 5.2% and 2.4% treatments (Tables 35 and 36). There was no significant difference in the response of the neutralized treatments or the non-neutralized treatments and the PO_4^{3-} deficient control treatment. Growth in the PO_4^{3-} deficient media was approximately 50% of the growth observed in the complete media control.

TABLE 34. ANALYSIS OF VARIANCE (ANOVA) OF STIMULATION TESTS WITH WP-F

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Prob.
Mean	5193112.52596	1	5193112.52596	506.00	0.0000
Phosphorus Level	76029.27979	4	192257.31995	18.73	0.0000
Neutralization	83027.44604	1	83027.44604	8.09	0.0075
Phosphorus vs. Neutralization/Non-neutralization	561676.41932	4	140419.10483	13.68	0.0000
Error	307889.54923	30	10262.98497		
Interactions					
Time	3807459.66365	4	951864.91591	106.49	0.0000
Time vs. Phosphorus	1081407.70097	16	67587.98131	7.56	0.0000
Time vs. Neutralization	120239.50460	4	30059.87615	3.36	0.0120
Time x Phosphorus x Neutralization	1075014.35844	16	67188.39740	7.52	0.0000
Error	1072640.32895	120	8938.66941		

TABLE 35. F-VALUES FOR COMPARISONS BETWEEN ALL POSSIBLE PAIRS OF MEANS AT 0, 24, 48, 72, AND 96 HOURS FOR WP-F ALGAL STIMULATION TESTS

Treatment Contrast	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
10% Non-Neut. - 10% Neut	0.00	0.00	1.73	20.42	59.69
7.0% Non-Neut. - 7% Neut	0.00	0.17	0.74	22.50	7.72
5.2% Non-Neut. - 2.4% Neut	0.00	0.02	0.60	26.97	18.83
2.4% Non-Neut. - 5.2% Neut	0.00	0.00	0.02	20.06	20.43
P0 ₃ Def. Control - 10% Non-Neut	0.00	0.62	2.43	9.26	20.99
P0 ₄ Def. Control - 7% Non-Neut	0.01	0.61	1.94	8.23	19.52
P0 ₃ Def. Control - 5.2% Non-Neut	0.00	0.36	0.55	0.24	10.79
P0 ₄ Def. Control - 2.4% Non-Neut	0.01	0.31	0.08	54.62	35.85
10% Non-Neut - 7% Non-Neut	0.00	0.00	0.03	0.03	0.03
10% Non-Neut - 5.2% Non-Neut	0.00	0.03	0.66	6.51	61.86
10% Non-Neut - 2.4% Non-Neut	0.01	0.05	1.62	108.85 ^a	111.69 ^a
7.0% Non-Neut - 5.2% Non-Neut	0.00	0.03	0.42	5.65	59.33
7.0% Non-Neut - 2.4% Non-Neut	0.00	0.05	1.22	105.24 ^a	108.28 ^a
5.2% Non-Neut - 2.4% Non-Neut	0.01	0.00	0.21	62.13	7.31
P0 ₃ Def. Control - 10% Neut	0.00	0.56	0.06	2.18	9.89
P0 ₄ Def. Control - 7% Neut	0.00	0.13	0.29	3.52	2.69
P0 ₃ Def. Control - 2.4% Neut.	0.00	0.23	0.00	22.11	1.11
P0 ₄ Def. Control - 5.2% Neut.	0.00	0.27	0.20	8.48	2.15
10% Neut. - 7% Neut.	0.00	0.15	0.09	0.16	22.90
10% Neut. - 2.4% Neut.	0.00	0.07	0.08	10.40	17.64
10% Neut. - 5.2% Neut.	0.00	0.05	0.04	2.06	2.81
7% Neut. - 2.4% Neut.	0.00	0.01	0.32	7.99	0.34
7% Neut. - 5.2% Neut.	0.00	0.02	0.01	1.07	9.66
2.4% Neut. - 5.2% Neut.	0.00	0.00	0.23	3.21	6.36

^a Significant at $p < .05$ using Scheffé's test with error rate adjustment for simultaneous comparisons.

TABLE 36. RESPONSE OF *SELENASTRUM CAPRICORNUTUM* IN ALGAL STIMULATION TESTS WITH WP-F SMOKE-DOSED PO_4^{3-} -DEFICIENT MEDIA (96-HR DATA)

Treatment	Nominal Total P (mg/L)	PO_4^{3-} (mg/L)	Initial pH	Mean (± 1.0 S.D.) Cell Counts ($\times 10^5/\text{mL}$)	Ratio Cell Counts to PO_4^{3-} Deficient Control	Mean (± 1.0 S.D.) Chlorophyll A ($\mu\text{g/L}$)
-----Non-Neutralized-----						
10% Stock	4.28	3.41	4.8	0.24 ± 0.11	0.07	1.71 ± 0.86
7% Stock	3.0	2.85	5.5	0.48 ± 0.36	0.15	3.04 ± 2.53
5.2% Stock	2.23	2.08	6.0	4.95 ± 2.96	1.52	101.3 ± 83.0
2.4% Stock	1.03	0.89	6.6	6.28 ± 3.83	1.93	62.9 ± 34.2
-----Neutralized-----						
10% Stock	4.28	3.83	7.35	5.04 ± 3.83	1.55	77.1 ± 50.8
7% Stock	3.0	2.70	7.35	1.97 ± 0.90	0.61	33.7 ± 23.4
5.2% Stock	2.23	2.05	7.35	2.37 ± 1.40	0.73	34.9 ± 22.1
2.4% Stock	1.03	1.20	7.35	3.73 ± 1.50	1.15	44.0 ± 8.58
-----Control-----						
PO_4^{3-} -deficient media	--	0.20	7.5	3.25 ± 1.29	--	17.67 ± 8.48
Complete media	--	--	7.38	6.55 ± 2.77	2.02	46.87 ± 16.69

One difference in the two algal stimulation tests was that the neutralized solutions showed greater growth than the phosphate deficient controls in the RP-BR test, but not in the WP-F test. This discrepancy may be related to the fact that PO_4^{3-} levels in the WP-F tests were saturating while PO_4^{3-} levels in the RP-BR test were not.

Phase II Acute Toxicity Tests. The responses of fish and macro-invertebrates to smoke-dosed river water were summarized as LC50 values as a function of initial pH and total phosphorus. These results are summarized in Table 37 for RP-BR and WP-F tests. Additional monitoring data for toxicity tests is summarized in Appendix C. The relationship between LC50 values based on initial pH and total phosphorus are relatively consistent. In the WP-F tests, the LC50 value based on phosphorus for Hexagenia was

TABLE 37. MEDIAN LETHAL CONCENTRATION (LC50) VALUES OF WP-F AND RP-BR ACUTE TOXICITY TESTS

Test Species	Test Duration	Total Phosphorus (µg/mL)		pH	
		LC50	95% Confidence Interval	LC50	95% Confidence Interval
-----White Phosphorus-----					
<u>D. magna</u>	48 hr	29.20	(25.64 - 33.25)	5.52	(5.28 - 5.77)
<u>H. azteca</u>	48 hr	34.99	(33.77 - 36.27)	4.36	(4.21 - 4.52)
<u>Hexagenia</u>	48 hr	44.08	(43.28 - 44.89)	3.99	(3.92 - 4.06)
<u>P. dissimulus</u>	48 hr	41.72	(40.26 - 43.24)	3.68	(3.61 - 3.75)
Fathead minnow					
<u>P. promelas</u>	96 hr	36.99	(35.87 - 38.15)	4.45	(4.33 - 4.57)
Rainbow Trout					
<u>S. gairdneri</u>	96 hr	33.22	(32.39 - 34.08)	5.12	(5.04 - 5.20)
Bluegill sunfish					
<u>L. macrochirus</u>	96 hr	43.76	(42.93 - 44.61)	3.92	(3.86 - 3.98)
Channel Catfish					
<u>I. punctatus</u>	96 hr	41.59	(39.63 - 43.65)	4.27	(4.06 - 4.50)
-----Red Phosphorus-----					
<u>D. magna</u>	48 hr	29.69	(29.10 - 30.28)	5.40	(5.28 - 5.53)
<u>H. azteca</u>	48 hr	31.44	(29.93 - 33.02)	5.16	(4.90 - 5.44)
<u>Hexagenia</u>	48 hr	42.18		4.02	
<u>P. dissimulus</u>	48 hr	55.66	(52.46 - 59.06)	3.43	(3.31 - 3.55)
Fathead minnow					
<u>P. promelas</u>	96 hr	28.75	(27.91 - 29.61)	5.50	(5.41 - 5.60)
Rainbow trout					
<u>S. gairdneri</u>	96 hr	40.55	(39.13 - 42.03)	4.41	(4.31 - 4.51)
Bluegill sunfish					
<u>L. macrochirus</u>	96 hr	35.90	(33.83 - 38.11)	3.97	(3.92 - 4.01)
Channel Catfish					
<u>I. punctatus</u>	96 hr	45.05	(44.17 - 45.94)	3.79	(3.72 - 3.85)

^a No partial mortality, confidence intervals not calculated.

higher than H. azteca, but was reversed for pH. Similar contrasting relationships were observed in the RP-BR tests among S. gairdneri, I. punctatus and L. macrochirus. These contrasts point to the variability associated with the degree of acidification imparted by the phosphorus smoke between burns. Because of this variability, no particular species of fish can be judged to be more sensitive than another nor can RP-BR smoke be

judged to be more toxic than WP-F smoke. The difference in LC50 values between WP-F and RP-BR for S. gairdneri may be attributed to the fact that the fish tested with WP-F had just buttoned up and were probably more sensitive due to age differences.

Among the invertebrates, first instar D. magna were the most sensitive species. Although LC50 values were not determined during Phase I tests with D. magna in well water, the mortality patterns at 48 hr agree with the responses observed in river water. The LC50 values repeatedly fell between a pH of 5.0 and 6.0. The midge larvae P. dissimulus was the most resilient species tested. Variation in the ranking of LC50 values may be attributed to inconsistencies in the relationship between pH and total phosphorus among burns.

3.3 SGF NO. 2 FOG OIL

Aromatic and saturated components of three batches of fog oil were analyzed by GC and only minor differences were observed in the chromatograms. The solubility of the oil in water was very low. The composition of aromatic components found in OWMs of vaporized and bulk fog oil was different than what would have been predicted from their relative abundances in the whole oil. Filtration removed most of the dispersed oil in the OWMs. Vaporization increased the number of free radicals, but did not increase the levels of phenolic compounds. Photolysis increased the number of water soluble constituents and radically changed the physical properties of vaporized and bulk fog oil.

Toxicity testing in Phase I and II indicated that the fog oil was relatively non-toxic before and after vaporization. The primary hazard is the potential for aquatic organisms to become fouled when oil adheres directly to body surfaces or is entrained during filter-feeding. Photolysis increased the toxicity of the oil, presumably by increasing the aqueous solubility of the oil.

3.3.1 Fog Oil Characterization (Phase I)

The three batches of fog oil and OWMs prepared with vaporized and bulk lots of oil were characterized in Phase I. No major differences were found in the aromatic and saturated hydrocarbon content of three batches of fog oil. Oil water mixtures prepared with bulk and vaporized fog oil did not differ significantly. Infrared spectroscopy was selected as the method for monitoring total oil concentrations in fog oil-water mixtures. Saturated solutions of fog oil contained 190 to 350 µg/L total oil, that was composed primarily of low molecular weight aromatic compounds. Filtration removed about half of the aromatic hydrocarbons from the OWM.

Bulk Fog Oil

Aromatic and saturated hydrocarbon fractions of the three batches of fog oil were analyzed by high resolution capillary gas chromatography (GC).

No differences in chromatographic patterns of aromatic hydrocarbons were apparent among these three batches of oil. The two fog oils designated Batch 2 and Batch 3 (most recent batches) contained significant amounts of what appeared to be the branched-chain saturated hydrocarbons, pristane and phytane. The saturated hydrocarbon chromatograms also showed the presence of a large number of unresolved components containing approximately 15 to 27 carbon atoms. Aromatic hydrocarbon chromatograms showed the presence of a great many unresolved components (Figure 8). Chromatograms of vaporized fog oil were indistinguishable from bulk fog oil chromatograms. Phenol content of the oils was very low.

Bulk and Vaporized Oil-Water Mixtures

In conjunction with the analysis of fog oils, we selected the oldest batch (Batch 1) and prepared oil water mixtures (OWM) of the oil and the vaporized oil (FO-1). This batch of fog oil was used because it may have been more oxidized than the other batches and, hence, may have contained more water soluble components. One hundred milliliters of Batch 2 fog oil was gently mixed (by slow stirring) with 9.9 L of well water for 4 hr and allowed to stand for 1 hr. This mixing method has been used for other oils and synfuel materials at PNL and provides a source of comparison for SFG No. 2 fog oils. The oil-water mix was siphoned and used for chemical analysis and preliminary toxicity tests with *D. magna*. Both filtered (0.45 μ m at 10 psi) and unfiltered fractions were tested and analyzed. Similarly, a slow mix was prepared with 67 mL of vaporized Batch 1 fog oil in 6.7 L of well water.

Infrared Analysis. Filtered and unfiltered 500-mL samples of the OWM were acidified, extracted with CCl_4 , and analyzed for total oil concentration on the day the OWM was prepared and on each of the following 2 days. Filtered and unfiltered well water blank samples were also analyzed on each of the 3 days. Total oil concentrations in the OWM are shown in Table 38. Infrared scans of 73.8 $\mu\text{g/mL}$ and 14.7 $\mu\text{g/mL}$ standard CCl_4 solutions are shown in Figure 9, along with a CCl_4 extract of filtered and unfiltered OWM.

Unfiltered well water blanks consistently showed no absorbence in the C-H stretching frequency region. However, filtration of well water blanks introduced a contaminant into the water equivalent to about 140 $\mu\text{g oil/L}$ water (Figure 9). Concentrations of total oil in the aqueous samples showed no significant change with time from day 0 through day 2.

Both aromatic and saturated hydrocarbon fractions in vaporized and bulk Batch 1 fog oil OWMs were analyzed by high resolution capillary gas chromatography (GC) using flame ionization detection (FID) after separation into saturated and aromatic fractions (Warner 1976). Concentrations of normal saturated hydrocarbons were extremely low in the water (<1 $\mu\text{g/L}$); no attempt was made to measure them.

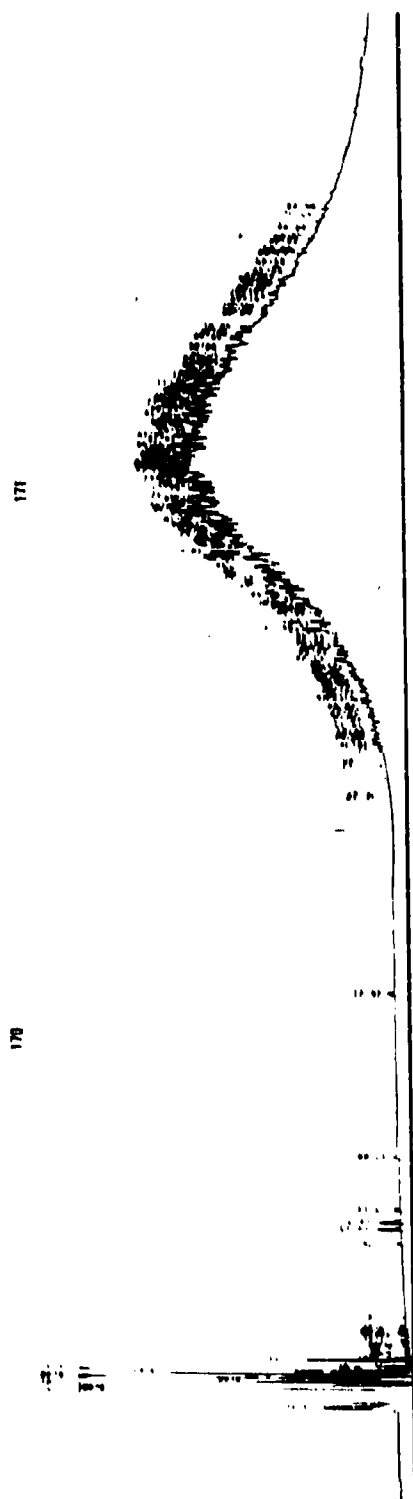


Figure 8. Gas chromatogram of a fog oil aromatic hydrocarbon fraction.

TABLE 38. INFRARED DETERMINATION OF FOG OIL CONCENTRATION OF OIL-WATER MIXTURES (OWM)

Sample	Fog Oil Concentration ($\mu\text{g/L}$)		
	Initial	24 hr	48 hr
Well Water			
Unfiltered	0	0	0
Filtered	160	110	150
Oil Water Mixtures			
Filtered A	210	270	210
Filtered B	190	200	250
Unfiltered A	260	160	(350) ^a
Unfiltered B	170	240	270
Unfiltered C	220	150	200
Unfiltered D	200	200	200
Unfiltered E	160	190	210
$\bar{X} \pm S$ (n = 5)	200 \pm 40	190 \pm 36	246 \pm 65 ^a

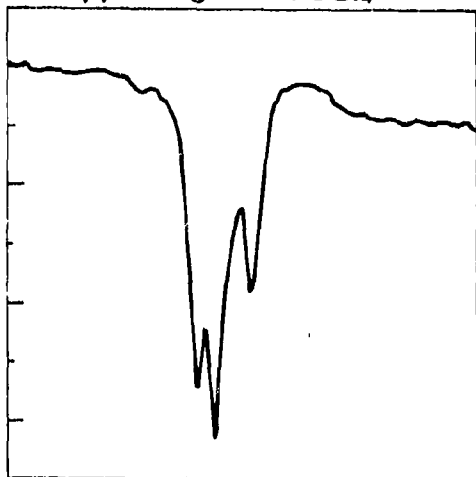
Summary

Oil in Filtered OWM: 220 \pm 31 $\mu\text{g/L}$ (n = 6)
 Oil in Filtered OWM after subtracting blank: ~80 $\mu\text{g/L}$
 Oil in Non-Filtered OWM: 200 \pm 36 $\mu\text{g/L}$ (n = 14)

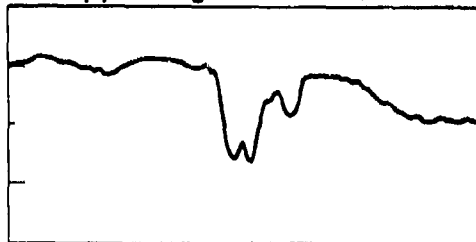
^a Mean concentration is 220 \pm 34 $\mu\text{g/L}$ for n = 4, deleting OWM A.

Chromatograms of the aromatic hydrocarbon fraction contained peaks with retention times corresponding to analytical standards of mono-, di-, and tri-aromatic hydrocarbons. A chromatogram of the aromatic hydrocarbons in an unfiltered OWM is shown in Figure 10. This OWM chromatogram is in marked contrast to that of the fog oil (Figure 8). The high molecular weight components contributing to the unresolved envelope are highly insoluble in water; hence, lower molecular weight components such as the benzenes, naphthalenes, and, to a lesser extent, the phenanthrenes are selectively incorporated into the aqueous phase of the OWM. These low molecular weight components are present only at very low concentration levels in the fog oil (<20 $\mu\text{g/g}$ oil) and are not observed in the oil chromatogram. Because they are readily water soluble, their presence becomes apparent in chromatograms of the OWM. Thus, the chemical composition of the fog oil OWM is very

73.8 ppm Fog 0.1 in CCl₄



14.7 ppm Fog Oil in CCl₄



CCl₄ Extract of 200 ppb OWM



CCl₄ Extract of 200 ppb Filtered OWM



CCl₄ Extract of Filtered Well Water

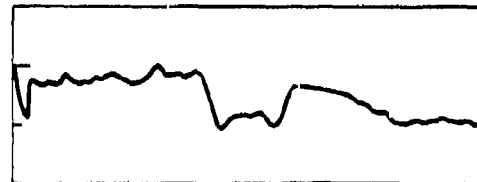


Figure 9. IR scans of selected OWM extractions and standard solutions of known amounts of fog oil in carbon tetrachloride. (See Figure 11 for scale on ordinate and abscissa).

different from that of the fog oil, and toxic properties of the OWM are likely to be substantially different from those which might be predicted on the basis of the bulk oil composition.

Filtered (0.45 μ m) OWM aromatic fractions showed no envelope of components, but some peaks corresponded to naphthalene, methylnaphthalenes, and dimethylnaphthalenes. Filtered OWM saturated hydrocarbon fractions showed no component envelope. The aromatic hydrocarbon fraction of an unfiltered vaporized oil fog OWM showed peaks corresponding to methyl substituted benzenes, naphthalenes, and methyl and dimethyl substituted naphthalenes. A trace of the envelope remained in the unfiltered OWM but was removed by filtration. Traces of mono- and di-aromatics remained in the filtered OWM. No phenols were detected in the filtered or unfiltered OWM samples.

Concentrations of various aromatic hydrocarbons and their alkyl substituted analogs in the OWM of bulk and vaporized Batch 1 fog oils were

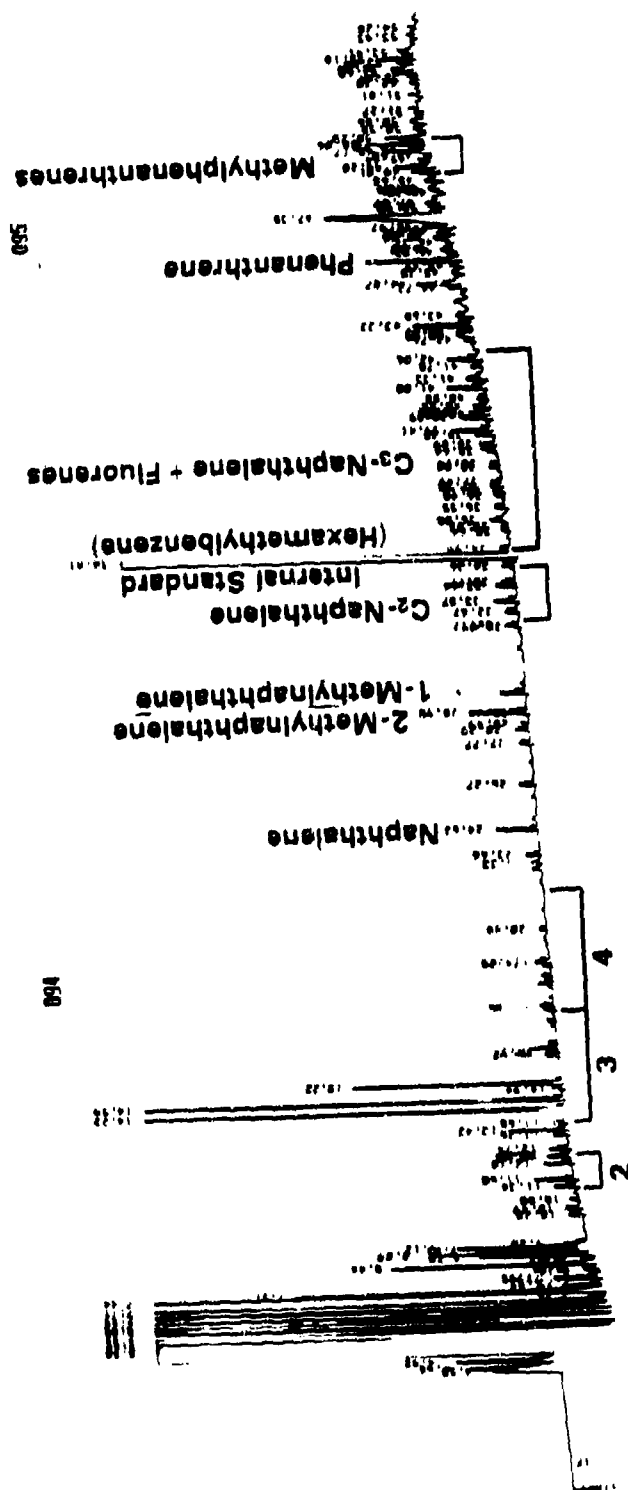


Figure 10. Chromatogram of the aromatic hydrocarbons from an unfiltered OMM of fog oil.

calculated (Tables 39 and 40, respectively). C₁ refers to methyl substitution, C₂ to dimethyl or ethyl, and so on. Distinct changes in the relative composition of total aromatic hydrocarbons were apparent in the extraction of bulk and vaporized Batch 1 OWM. Total aromatic hydrocarbon concentrations were reduced by nearly one half with filtration of the OWM through 0.45 µm "Millipore (Bedford, MA)" membrane filter. Further, concentrations of mono- and di-aromatics were increased relative to tri-aromatics by filtration.

Changes caused by filtration can be attributed to more efficient removal by the filter of the larger, higher molecular weight hydrocarbons that are more water insoluble and tend to be present as small droplets dispersed throughout the water rather than as individual molecules in true aqueous solution. Lesser amounts of the lower molecular weight aromatic hydrocarbons (benzenes and naphthalenes) are removed by filtration of the water because these components are present more in true solution and less in filterable oil droplets.

Aromatic hydrocarbon fractions of an unfiltered OWM showed the "unresolved envelope" chromatographic pattern found in the oil aromatic fraction was still present but concentrations of individual components were very low and peak retention times corresponding to naphthalene and the methyl naphthalenes had become apparent. The saturated hydrocarbon OWM fraction showed small amounts of components comprising the unresolved envelope in the oil fraction. No phenols were detected.

TABLE 39. BULK FOG OIL OWM AROMATIC HYDROCARBON CONCENTRATIONS (µg/L)

Aromatic Hydrocarbon	Unfiltered			Filtered		
	Rep 1	Rep 2	Mean ± Range	Rep 1	Rep 2	Mean ± Range
C ₂ Benzenes	1.0	1.0	1.0 ± 0.0	1.3	1.1	1.2 ± 0.18
C ₃ Benzenes	0.7	0.7	0.7 ± 0.0	0.7	1.0	0.8 ± 0.1
C ₄ Benzenes	0.7	1.2	1.0 ± 0.2	0.8	0.8	0.8 ± 0.0
Naphthalene	0.2	0.2	0.2 ± 0.0	0.1	0.2	0.2 ± 0.0
C ₁ Naphthalenes	0.3	0.4	0.4 ± 0.0	0.2	0.4	0.3 ± 0.1
C ₂ Naphthalenes	0.5	0.5	0.5 ± 0.0	0.04	0.3	0.2 ± 0.2
C ₃ Naphthalenes	1.6	1.7	1.6 ± 0.05	0.5	1.0	0.8 ± 0.26
Phenanthrene	0.5	0.5	0.5 ± 0.0	<0.1	0.1	<0.1
C ₁ Phenanthrenes	1.1	1.2	1.2 ± 0.0	<0.1	0.1	<0.1
Total	6.6	7.4	7.0 ± 0.4	3.6	5.0	4.3 ± 0.7

TABLE 40. VAPORIZED FOG OIL OWM AROMATIC HYDROCARBON CONCENTRATIONS (µg/L)

Aromatic Hydrocarbon	Unfiltered			Filtered		
	Rep 1	Rep 2	Mean ± Range	Rep 1	Rep 2	Mean ± Range
C ₂ Benzenes	0.7	0.7	0.7 ± 0.04	0.8	0.8	0.8 ± 0.04
C ₂ Benzenes	1.2	1.2	1.2 ± 0.00	0.9	1.0	0.9 ± 0.09
C ₃ Benzenes	3.9	4.2	4.1 ± 0.18	2.6	4.2	3.8 ± 0.82
Naphthalene	0.6	0.7	0.6 ± 0.04	0.5	0.5	0.5 ± 0.01
C ₁ Naphthalenes	1.0	1.3	1.2 ± 0.1	0.7	0.7	0.7 ± 0.0
C ₂ Naphthalenes	1.1	1.5	1.3 ± 0.28	0.4	0.7	0.5 ± 0.25
C ₃ Naphthalenes	1.8	2.1	1.9 ± 0.27	0.2	0.8	0.5 ± 0.43
Phenanthrene	0.5	0.5	0.5 ± 0.03	<0.1	<0.1	<0.1
C ₁ Phenanthrenes	0.9	0.8	0.8 ± 0.05	<0.1	<0.1	<0.1
Total	11.7	12.8	12.2 ± 0.6	6.1	9.1	7.6 ± 1.5

A second vaporization of Batch 1 fog oil (FO-3) was conducted at 6% excess air (~1.2% O₂). The oil was collected from a glass wool plug for analysis and toxicity testing. Some differences in composition of aromatic hydrocarbons were apparent between OWM generated from the vaporized and bulk fog oil. The bulk fog oil had a relatively higher proportion of C₂ and C₃ benzenes than the vaporized oil. This can be attributed to the loss of more volatile hydrocarbons to the atmosphere during the vaporization/condensation process. An apparent decrease in the contribution of C₃-naphthalenes after vaporization is not readily rationalized. There was a greater concentration of aromatics in the OWM prepared from the vaporized fog oil than in that from the bulk oil. The difference could be a result of slight changes in the physical properties of the oil (changes in viscosity, emulsifier content, etc.), or could be an uncontrollable variation in the preparation method. The total concentration of aromatic hydrocarbon was 32.9 ± 5.2 µg/L (Table 41). This total concentration is somewhat higher than the aromatic hydrocarbon concentration that was found in the prior OWMs. The high concentration was attributed to the presence of components that eluted in the same retention time window as a C₁-phenanthrene analytical standard.

Because the OWM samples were not filtered, the presence of undissolved oil droplets would account for the high concentrations of higher molecular weight hydrocarbons, the high total oil concentrations, and the unresolved envelopes in the saturated and aromatic hydrocarbon chromatograms. Individual saturated hydrocarbon concentrations in both the oil and its OWM were very low and they were not quantified. Phenol concentrations in both the oil and OWM were very low (0.17 ± 0.04 µg/g oil and 0.02 µg/mL OWM, respectively).

TABLE 41. VAPORIZED FOG OIL (FO-3) OWM AROMATIC HYDROCARBON FRACTION CONCENTRATION IN $\mu\text{g/L}$.

Hydrocarbon	Rep 1	Rep 2	Mean \pm range
C ₂ Benzenes	0.6	0.8	0.7 \pm 0.1
C ₃ Benzenes	0.6	0.8	0.7 \pm 0.1
C ₄ Benzenes	0.9	1.3	1.1 \pm 0.2
Naphthalene	0.4	0.5	0.5 \pm 0.0
C ₁ Naphthalenes	0.3	0.4	0.4 \pm 0.1
C ₂ Naphthalenes	0.4	0.6	0.5 \pm 0.1
C ₃ Naphthalenes	2.3	3.9	3.1 \pm 0.8
Phenanthrene	0.6	1.7	1.2 \pm 0.6
C ₁ Phenanthrenes	<u>21.7</u>	<u>28.1</u>	<u>24.9 \pm 3.2</u>
Total	27.8	38.1	32.9 \pm 5.2

3.3.2 Characterization of Photolyzed Fog Oils

Two experiments were performed with Batch 3 fog oil: a 24-hr and a 7 day photolysis exposure. Vaporized and bulk fog oil were exposed in each experiment. Photolysis caused distinct changes in the physical properties of the oil. The formation of free radicals in the oil resulted in the formation of several classes of oxidation products. Infrared (IR) spectra of the photolyzed oil indicated increases in the content of carbonyl oxygen and possibly carboxylic acids. GC/MS analysis of OWMs prepared from the photolyzed fog oil revealed the presence of ketones.

Photolysis of Fog Oil

Photolysis of fog oil was monitored by visual inspection and by determining the number of free radicals generated by the radiation. Monitoring of free radicals was performed with a Varian electron-spin resonance spectrometer, calibrated with the stable free radical diphenylpicrylhydrazyl. The initial free radical concentration in the vaporized oil was higher than in the unvaporized oil, which is indicative of oxidative or pyrolytic reactions (Table 42). As exposure continued, both oils became increasingly yellow, and after 3 hr became turbid. Free radical concentration increased steadily in the case of the unvaporized oil, but rose and then fell again in the vaporized oil before rising steadily like the unvaporized oil. Radical concentrations in both oils after 7 days were similar, although somewhat higher in the unvaporized oil. After 24 hr, the color was orange.

TABLE 42. FREE RADICAL CONCENTRATIONS IN PHOTOLYZED FOG OILS

Time of Exposure	Fog Oil (10^{14} radicals/g)	Vaporized Fog Oil (10^{14} radicals/g)
0	1.2	6.7
10 min	1.2	8.9
20 min	1.6	--
30 min	2.1	10.1
60 min	3.4	6.4
120 min	9.8	--
180 min	13.6	6.4
270 min	13.7	--
360 min	--	10.1
24 hr	16.5	33.7
7 days ^a	68.1	56.8

^a Separate exposure.

From the absorbance at 300 nm (200 nm for unvaporized fog oil, 244 nm for vaporized), it can be calculated that one half the light at this wavelength would be absorbed within the first 1 to 2 microns of the oil surface.

Infrared and Ultraviolet Absorbance

The infrared spectra of thin films of photolyzed and unphotolyzed oils were obtained in order to obtain an estimate of oxygen incorporation in the form of hydroxy or carbonyl groups. Figures 11 and 12 present scans from 4000 to 1200/cm of the bulk Batch 3 fog oil and the corresponding vaporized oil. The scans are almost identical, with the exception that there is slightly higher absorbance in the 1730/cm region for the vaporized oil. Figures 13 and 14 show the absorbance of a vaporized oil subjected to photolysis for 24 hr, compared to bulk oil amended with 10% cyclohexanone. The percent carbonyl oxygen in the amended sample is calculated to be 1.6%. It is thus apparent that the concentration of carbonyl oxygen in the non-photolyzed sample is on the order of a few hundred mg/L. Throughout the rest of the spectrum, there is no discernible difference between photolyzed and photolyzed oils. No increase in the absorption at 3600/cm (hydroxyl stretching frequency) was noted. The -OH absorbances in Figures 11 thru 14 in this region are probably due to water in the air.

Oil photolyzed for 7 days exhibited pronounced changes in infrared absorbance in two regions: the carbonyl region (1650 to 1850/cm), and hydrogen-bonded proton region (3200 to 3600/cm). Figure 15 shows the

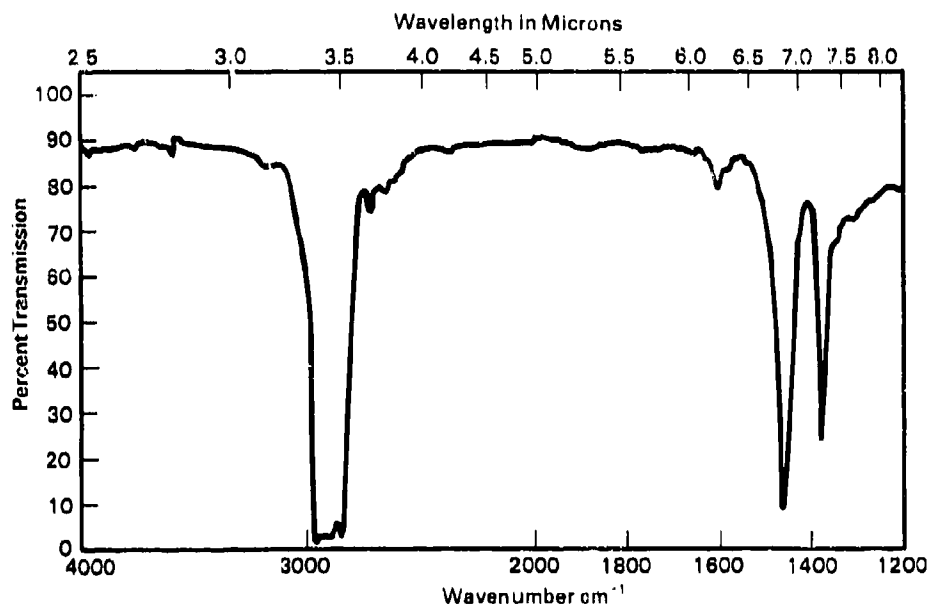


Figure 11. Infrared scans of thin films of Batch 3 bulk fog oil.

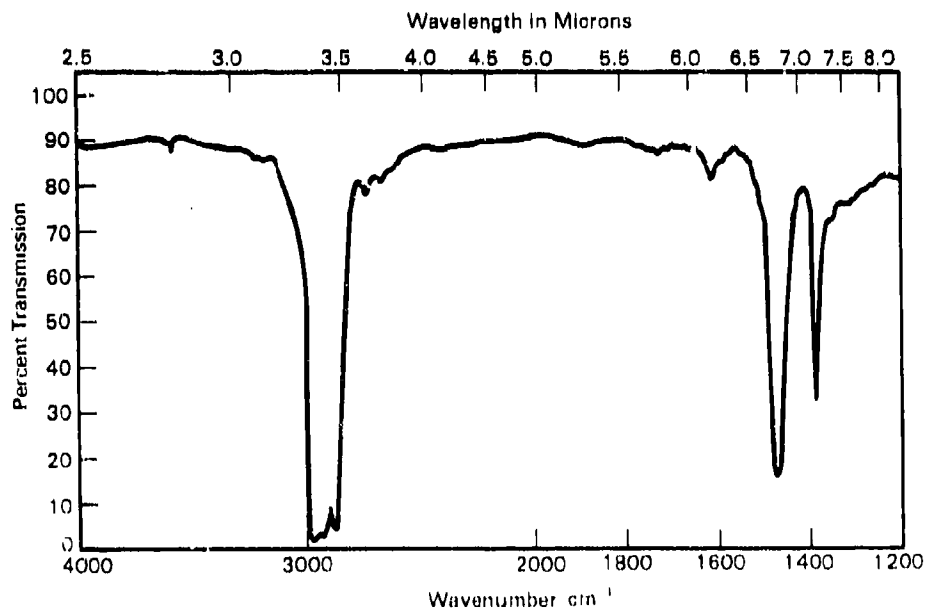


Figure 12. Infrared scan of thin film of Batch 3 vaporized fog oil.

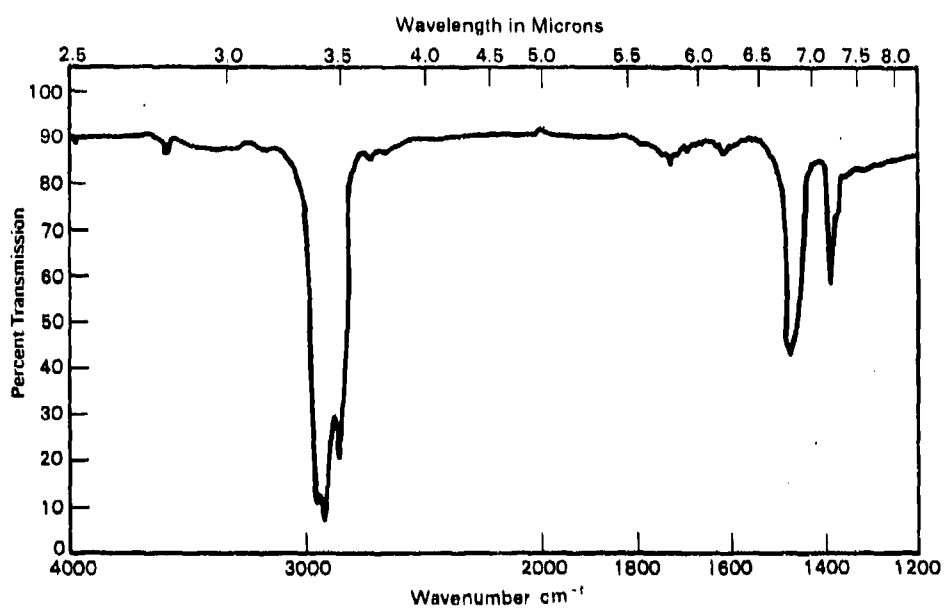


Figure 13. Infrared scans of thin films of fog oil.

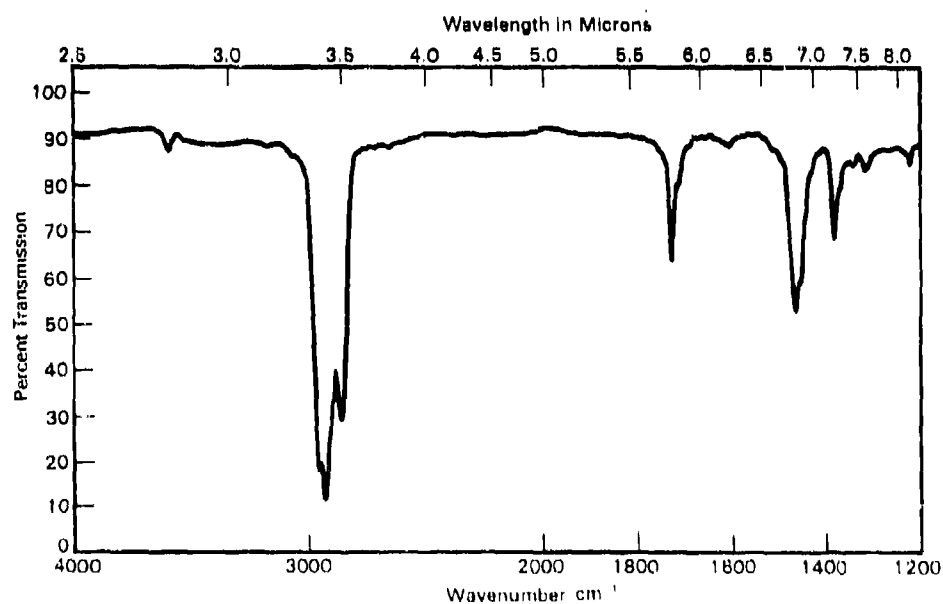


Figure 14. Infrared scan of thin film of untreated fog oil plus 10% cyclohexanone.

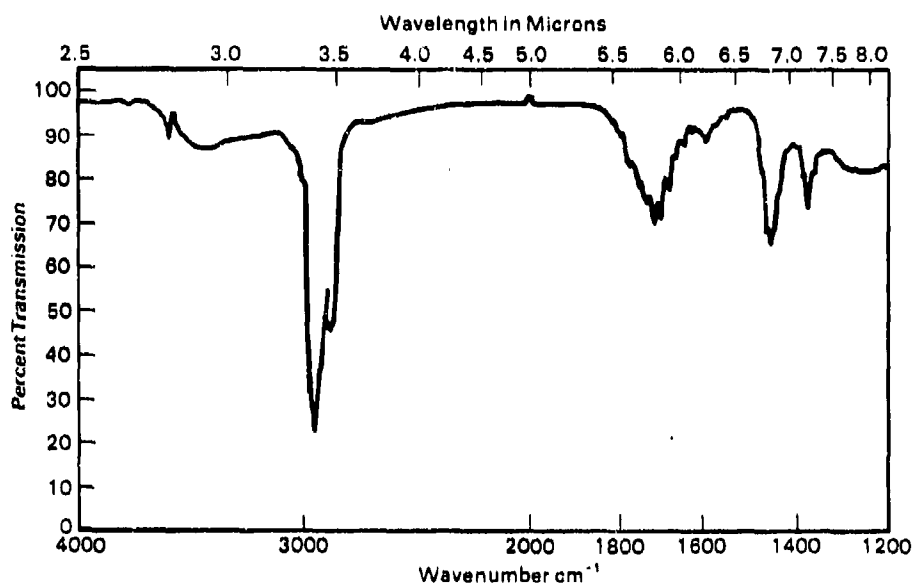


Figure 15. Infrared scan of thin film of Batch 3 fog oil photolyzed for 7 days.

spectrum taken from a thin film of 7-day photolyzed fog oil. The carbonyl absorbance is significant when compared to the sample amended with cyclohexanone (Figure 14). Because the carbonyl absorbance band is broad, a quantitative estimate of carbonyl oxygen is not possible; however, it is probable that concentration in the oil is several percent. The enhanced absorbance in the H-bonding region indicates that carboxylic acids may also be present in the 7-day photolyzed oil, since these compounds exhibit this absorbance behavior (Silverstein et al. 1974). Infrared scans of thin films of unvaporized and vaporized fog oils after 7 days of photolysis were identical.

Comparison of ultraviolet absorbances exhibited by unphotolyzed and photolyzed oils showed pronounced differences only in the case of the 7-day sample. A shift in absorption maximum from 217 nm to 212 nm was observed, with the appearance of a new absorbance shoulder at 240 to 248 nm. This absorbance is consistent with the presence of alpha, beta-unsaturated ketones and with acetophenones (Silverstein et al. 1974). Carboxylic acids, indicated to be present from the infrared scan, would also contribute to the absorbance in this region. Benzoic acid, for example, has an absorbance at 230 nm.

Gas Chromatographic Analysis of OWM of Photolyzed Oils

Water extraction of 24-hr photolyzed oils was conducted with vigorous stirring, and oil concentrations in water were higher than previous extracts used for toxicity testing. Isooctane extracts of 20 mL of the water-soluble fractions, initially used for determination of total oil, were examined by gas chromatography. Chromatograms obtained of vaporized and 14 hr photolyzed/vaporized fog oil using nonpolar bonded phase capillary columns are presented in Figures 16 and 17, respectively. The photolyzed material (Figure 17) contains more lower molecular weight material, as indicated by the somewhat higher peaks at early retention times. The column used would not elute highly polar compounds (carboxylic acids, alcohols, etc.). The infrared scans discussed above indicate that concentrations of polar compounds in the 24-hr photolyzed bulk oil are low. However, aqueous extraction would tend to selectively remove polar materials, and therefore they should be a significantly higher percentage of the water soluble fraction. The unresolved envelopes of material that are the predominant features of the chromatograms in Figure 16 and 17 indicate that, for the most part, the material extracted into the water for this experiment consisted of unchanged hydrocarbons.

When the water soluble fraction of unvaporized, 24-hr photolyzed oil was analyzed by capillary gas chromatography using a mass-selective detector (Hewlett-Packard Model 5970A), seven components were found in the early retention fraction of the photolyzed oil that were not present in the unphotolyzed oil. The identity of these compounds could not be established, because the components were not resolved sufficiently from other oil compounds to yield unambiguous spectra. The concentrations of these in the water were at most in the $\mu\text{g/L}$ range. The changes brought about by photolysis for 24 hr, while readily apparent to the eye, apparently did not profoundly affect the overall chemical composition of the water soluble extract. However, small changes in composition can have significant effects on the physical properties of the oil by producing changes in surface tension and water miscibility and emulsification.

The changes in the infrared spectrum of the 7-day photolyzed oils indicated significant amounts of oxygenated material had been formed. Therefore, water-soluble fractions used for toxicity testing were examined for components containing highly polar hydroxyl groups, using a derivatization procedure. In addition, hexane extracts of the water samples were directly injected into the gas chromatograph.

Samples of water used for toxicity testing (50 mL), were extracted twice with 10 mL hexane. The extracts were concentrated to 1 mL in n-heptane. The extracts were injected onto a 60 m capillary column in a gas chromatograph programmed from 70° to 250°C at 4°C/min. Detection was with a mass-selective detector, scanning from mass 40 to 300. A total ion chromatogram obtained from the vaporized, 7-day photolyzed aqueous extract is presented in Figure 18. Tentative assignment of structure has been made for several of the major components in the chromatogram.

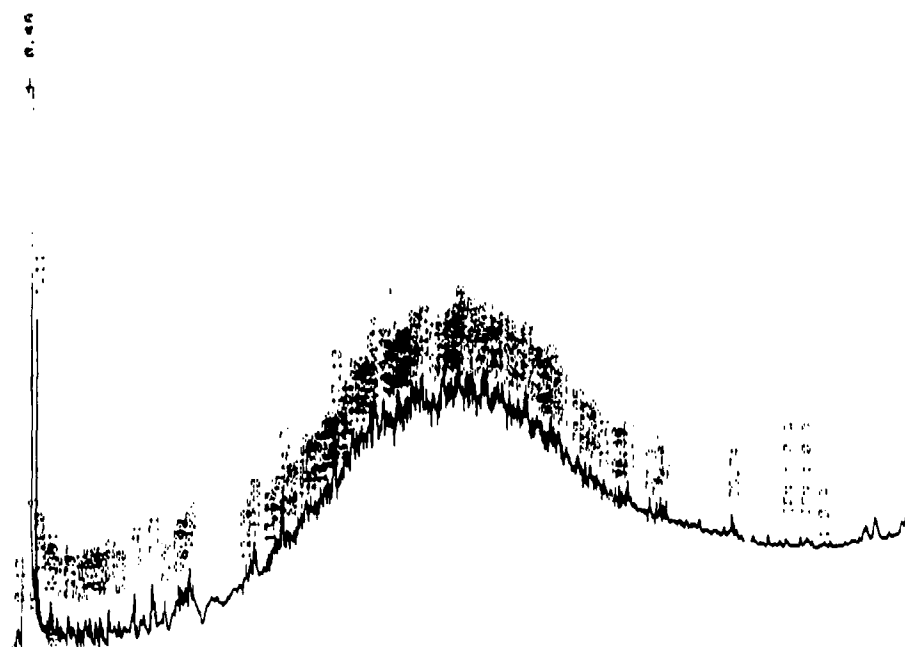


Figure 16. Capillary gas chromatogram of isooctane extract of water soluble fraction of vaporized fog oils.

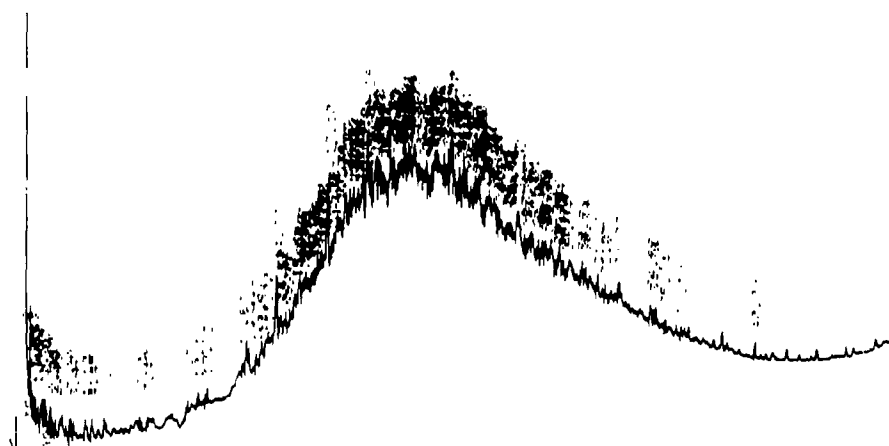


Figure 17. Capillary gas chromatogram of isooctane extract of water soluble fraction of 24-hr photolyzed/vaporized fog oil.

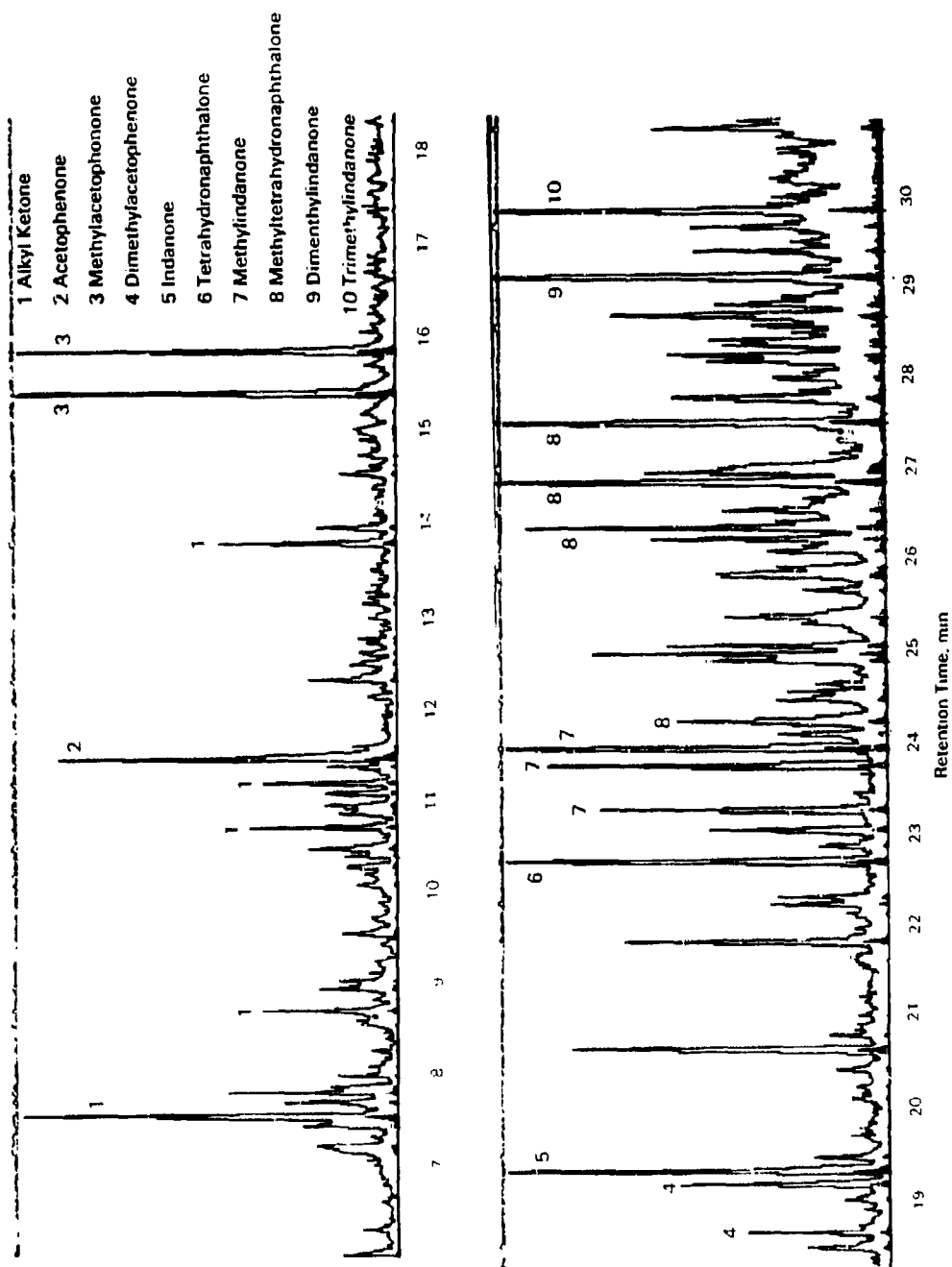


Figure 18. Total ionization chromatogram of hexane-extractable components from water soluble fraction of vaporized, 7-day photolyzed fog oil.

Initial structural assignments were made by referring to the "Eight Peak Index" (Mass Spectrometry Data Centre 1974), and comparing with published spectra of related compounds found in the EPA/NIH Mass Spectral Data Base (Heller and Milne 1978). Identification of exact structural isomers requires comparison of spectra and retention time with authentic standards. It is stressed that without such confirmation, the proposed structures are tentative. They are consistent, however, with the absorption spectra for the 7-day photolyzed fog oils discussed above. Chromatograms obtained from both vaporized and unvaporized 7-day photolyzed oil test water were essentially the same.

The hexane extract obtained from the stock solutions of unphotolyzed oil gave a chromatogram with no evidence of ketones, or of hydrocarbons related to fog oil. In the water from the previous experiment involving 24-hr photolysis, an unresolved envelope indicative of fog oil was present in the gas chromatogram. In the case of the 7-day photolysis, stirring was much less vigorous. Conditions were such that only truly water-soluble components were solubilized.

The hexane extracts were next treated with a powerful derivatizing agent, Sylon BTZ, that will form silyl ethers from hydroxylated (alcohol) compounds. The concentrated hexane extracts were treated with 0.1 mL of Sylon BTZ, left overnight, and the excess reagent decomposed with 3% ammonia. The treated samples were chromatographed as described above for the underivatized extracts. Components emerging from the column were monitored using the mass-selective detector. The detector was set to detect all components giving ion fragments of $M = 73$ and $M = 45$. These ions are characteristic of silyl ethers. A comparison of a single ion chromatogram obtained from the photolyzed sample, with that from the unphotolyzed sample showed no substantial difference between them. It is concluded from this experiment that compounds containing hydroxyl groups were not present in the water from the photolyzed oil in any excess over that from the unphotolyzed oil.

Although there is infrared evidence for the presence of carboxylic acids in the 7-day photolyzed oil, we did not investigate the possibility that carboxylated compounds were present in the aqueous extracts used for toxicity testing. They would not have been detected using the methods described above, and may not have been extracted from the water with hexane under the conditions used. Since carboxylic acids could have considerable influence on toxicity, as well as on the physical properties of the OWM, further work should be conducted to determine whether these compounds contribute substantially to the composition of the water-soluble fraction from photolyzed oils.

3.3.3 Evaluation of Other Methods for Monitoring OWMs

Total organic carbon and liquid chromatographic methods were investigated for monitoring OWMs and were found to be unsuitable. Measurement of

total organic carbon (TOC) has been successfully employed as a monitoring tool in previous studies of suspensions of coal liquids in water (Bean et al. 1981). The use of TOC measurement as a rapid oil monitoring tool was briefly investigated. The incentive for use of TOC is that results can be obtained very rapidly. However, the data presented in Table 43 demonstrate that TOC cannot be used to monitor oil concentrations effectively in Fog Oil OWMs. Total carbon (TC) was composed primarily of carbonate carbon, which was removed from the sample by acidification and sparging the sample with oxygen gas. The carbon remaining after removal of carbonate is designated TOC.

The problem with using TOC was that the background levels of organic carbon are about 3 mg/L, which are an order of magnitude higher than levels of oil as measured by IR (0.2 mg/L). Therefore, no difference in TOC concentration could be detected between well water samples and OWM samples.

Liquid Chromatography

A brief investigation of liquid chromatography to determine total oil in the OWM was also conducted. Injection of up to 200 mL of the OWM onto a reverse-phase bonded n-octadecane column did not produce an absorbance envelope which could be quantitated. Both methanol and acetonitrile were used as eluting solvents without positive results. It was concluded that an LC method, although feasible, would be difficult to develop for OWMs having oil concentrations much lower than 1 mg/L, and would have little advantage over the infrared absorbance method.

3.3.4 Toxicity of SGF No. 2 Fog Oil

Preliminary toxicity tests with D. magna were performed on oil-water dispersions of bulk and vaporized fog oil during Phase I of the project. During Phase II, screening toxicity tests were performed with the green alga S. capricornutum, P. promelas, and mayfly larvae (Hexagenia sp.) with

TABLE 43. MEASURED TOTAL ORGANIC CARBON ($\mu\text{g/L}$) IN FILTERED AND NON-FILTERED OIL/WATER MIXTURES AND WELL WATER SAMPLES

	Initial	24 hr	48 hr
Well Water ^a	3.20 \pm 0.76	2.24 \pm 0.31	2.25 \pm 0.28
Well Water, Filtered	3.10 \pm 0.39	--	--
OWM	3.70 \pm 1.95	2.73 \pm 0.22	2.16 \pm 0.18
OWM, Filtered	3.28 \pm 0.37	2.94 \pm 0.16	2.83 \pm 0.28

^a Used to prepare the OWM from Batch 1 fog oil.

three batches of SGF No. 2 fog oil. Batch 3 was selected for additional studies to determine the effect of vaporization and photolysis on the chemistry and toxicity of the fog oil. Toxicity testing continued in Phase II with P. promelas, D. magna, P. dissimilis and H. azteca.

Phase I Tests

Preliminary toxicity tests were performed with filtered (0.45 μ m) and unfiltered OWMs prepared from a 30-L standard slow mix at 174 rpm. In this initial mix, the plastic propeller was positioned 15 cm below the surface of the water. Vaporized (FO-1) and unvaporized Batch 1 fog oils were used in the slow mix preparations. Exposure solutions were prepared with 10% to 100% of the filtered and unfiltered OWM for the vaporized and bulk Batch 1 fog oil. There was no mortality over 96 hr in any of the treatments of the filtered OWM for either vaporized or bulk fog oil. In tests with the unfiltered OWM, the exposed organisms immediately floated to the surface; floating appeared to be dose-dependent (Table 44). After 24 hr, "floaters"

TABLE 44. SUMMARY OF MORTALITIES (%) AND FLOATERS (%) FROM SCREENING TOXICITY TESTS OF BULK AND VAPORIZED (FO-1) BATCH 1 FOG OIL^a

Dilution	2.5 hr	24 hr		48 hr			72 hr			96 hr		
	Floaters ^b	Floaters		Floaters			Floaters			Floaters		
		Dead	Alive	Mort.	Dead	Alive	Mort.	Dead	Alive	Mort.	Dead	Alive
-----Bulk-----												
Control	4.2	0	0	0	0	0	0	0	0	0	0	0
10%	4.2	0	0	0	0	0	0	0	0	0	0	0
20%	12.5	0	0	0	0	0	0	0	0	4.2	0	0
40%	58.3	0	41.7	0	0	25.0	4.2	0	25	66.7	20.8	0
60%	58.3	0	75.0	0	0	62.5	41.7	33.3	29.2	95.8	66.7	0
100%	100	0	100	0	0	100	50.0	50.0	45.8	95.8	95.8	0
-----Vaporized-----												
Control	0	0	0	0	0	0	0	0	0	0	0	0
10%	0	0	0	0	0	0	0	0	0	0	0	4.2
20%	4.2	0	8.3	0	0	4.2	0	0	4.2	25.0	12.5	0
40%	50.0	0	25.0	0	0	20.8	33.3	20.8	4.2	100	29.2	0
60%	66.7	0	20.8	0	0	29.2	29.2	8.3	12.5	100	12.5	0
100%	70.8	0	33.3	0	0	37.5	12.5	12.5	29.2	87.5	37.5	0

^aMortality columns contain dead floaters and dead instars lying on the bottom of exposure beakers.

^bAll floaters at 2.5 hr were alive.

persisted in the 20% and higher treatments for the duration of the test. Mortality was not observed until 72 hr. The floating phenomenon resulted from either adherence of oil to the organism's carapace or internal accumulation of microdroplets by filtration.

The OWM were initially set up to test extraction and analytical procedures. The preliminary analysis for total oil in the stock solutions yielded conflicting results, and the concentration of total oil in the OWMs was believed to be 0.2 to 0.3 mg/L. The mortality observed in the tests can be attributed to the physical stress of floating, chemical toxicity of the oil, starvation, or interactions from any combination of these factors. Comparatively, there were more floaters in the bulk oil test than in the vaporized fog oil test. Uncertainties in the amount of oil present in the stock solutions prevent a direct comparison of the toxicity of the vaporized and bulk Batch 1 fog oil.

A second 48-hr screening toxicity test was prepared in Phase I with vaporized Batch 1 fog oil that had been collected on a glass wool plug (FO-3). The slow mix was modified to accommodate the smaller volume of vaporized oil available for testing. The ratio of oil to water was kept constant (1:99), and the propeller blades were positioned 2.5 cm below the surface of the water. Positioning the propeller closer to the surface of the water created more mixing energy and, therefore, dispersed more oil into the stock solutions. The resulting concentration of total oil (by IR) in the stock solution was 1.8 mg/L, roughly 10 times higher than that of the stock solution of the initial screening tests. Floaters were more prevalent in all the concentrations tested (Table 45). It is not surprising that some degree of mortality was associated with the 10% solutions at 48 hr since total oil concentrations in the 10% exposure solutions were comparable to

TABLE 45. MORTALITY DATA OF *D. MAGNA* EXPOSED TO OWM OF BATCH 1 VAPORIZED FOG OIL (FO-3)

% Stock	Oil mg/mL	24 hr ^a		48 hr ^a	
		Alive	Dead	Alive	Dead
100	1.8	20	0	5	15
60	1.1	19	1	7	13
40	0.8	20	0	13	7
20	0.4	21	0	12	9
10	0.2	20	0	12	8
Control	0	20	0	20	0

^a All test organisms were floating except controls.

the stock solution of the initial test. Oil slicks had developed after 24 hr in the more concentrated exposure solutions, indicating that suspended oil droplets in the stock solution were not in equilibrium with the truly dissolved fog oil. It was not possible to ascertain whether or not the fog oil that had vaporized in the presence of ~1.2% O₂ was more toxic than the initial FO-1 vaporized fog oil. The comparison was not possible because of differences in mixing and the amount of oil in the stock solutions. Interpretation of toxicity results was complicated by the presence of excessive amounts of oil, which made it difficult to separate chemical toxicity from physical effects of the oil.

Phase II Tests

Phase II screening toxicity tests were performed with P. promelas, Hexagenia sp. and the green alga S. capricornutum. Hexagenia was tested because its relatively large size would minimize the potential for physical effects of the oil interfering with the interpretation of test results. Moreover, the large surface area of exposed abdominal gills would predisposed this species to absorption of dissolved fog oil. Consequently the species intuitively could be one of the more sensitive aquatic species available for testing.

The results of screening toxicity tests with P. promelas and Hexagenia are summarized in Tables 46 and 47, respectively. Generally, the stock solutions generated from the fish tests contained less total fog oil at the start of the tests than the stock solutions generated for testing Hexagenia. After 24 hr, fog oil slicks were visible in the 10% and 100% exposure solutions indicating that the amount of oil present at the start of the tests exceeded the solubility of fog oil in water. This suggests that soluble components of the fog oil were not present in sufficient quantities to be toxic. Dissolved oxygen did not decrease over the 96 hr duration of the P. promelas tests indicating that the fog oil has very low potential for creating a chemical oxygen demand. Based on a lack of dose dependent toxicity in these screening tests, the toxicity of the three batches of fog oil were judged to be relatively non-toxic to invertebrates and fish.

The algal toxicity tests with S. capricornutum pointed towards an indication of toxicity in the more concentrated exposure solutions. Comparatively, Batch 1 and Batch 2 tests showed significant growth inhibition at 96 hr. There was an indication that the lower exposure concentrations of fog oil may have stimulated algal growth, but the difference in mean cell counts of these treatments were equivalent to cell counts in the control treatments (Table 48). In all algal tests, differences in treatments did not become apparent until 96 hr of exposure. With the exception of the Batch 1 test, growth inhibition was either not significant or was less than 50% at 96 hr in all tests with bulk and vaporized fog oil (FO-4). Consequently, EC50 concentrations were not calculated.

TABLE 46. SUMMARY OF 96-HR BATCH SCREENING TOXICITY TESTS WITH
PIMEPHALES PROMELAS

Treatment	Total Oil (mg/L)	Mortality	96-hr Data Combined	D.O. (mg/L)
-----Batch 1-----				
Control	0.16	0/8		7.7
Control	0.16	0/8	0%	8.0
1%	0.16	0/8		7.7
1%	0.16	0/8	0%	7.6
10%	0.55	0/8		8.0
10%	0.55	0/8	0%	7.5
100%	1.11	0/8		7.7
100%	1.11	0/8	0%	7.8
-----Batch 2-----				
Control	0.05	1/10		Not Measured
Control	0.05	0/10	5%	Not Measured
100%	0.64	0/10		Not Measured
100%	2.37	0/10	0%	Not Measured
-----Batch 3-----				
Control	0.05	0/10		Not Measured
Control	0.05	0/10	0%	Not Measured
100%	0.83	0/10		Not Measured
100%	0.45	0/10	0%	Not Measured

TABLE 47. SUMMARY OF 48-HR SCREENING TOXICITY TESTS WITH HEXAGENIA

Treatment	Total Oil (mg/L)	Mortality	48-hr Data Combined	D.O. (mg/L)
-----Batch 1-----				
Control	0	2/10		7.1
Control	0	1/10	15%	7.0
10%	0.58 ± 0.02	1/10		7.5
10%	0.55 ± 0.00	1/10	10%	7.4
100%	5.22 ± 0.10	1/10		7.9
100%	5.17 ± 1.10	0/10	5%	7.5
-----Batch 2-----				
Control	0	1/10		7.6
Control	0	0/10	5%	7.7
10%	0.60 ± 0.02	9/10		7.6
10%	0.69 ± 0.04	0/10	0%	7.7
100%	5.51 ± 0.65	1/10		7.4
100%	5.87 ± 0.87	0/10	0%	7.5
-----Batch 3-----				
Control	0	0/10		7.4
Control	0	0/10	0%	7.5
10%	0.32 ± 0.01	0/10		7.6
100%	2.94 ± 0.12	0/10	0%	7.5
10%	0.35 ± 0.02	0/10		7.8
100%	3.46 ± 0.31	0/10	0%	7.4

TABLE 48. COMPARISON OF ALGAL TOXICITY OF THREE BATCHES OF SGF NO. 2 FOG OIL BASED ON THE 96-HR MEAN CELL COUNTS

Treatment	Batch 1		Batch 2		Batch 3	
	Total Oil (mg/L)	Mean Cell Counts ^a	Total Oil (mg/L)	Mean Cell Counts ^a	Total Oil (mg/L)	Mean Cell Counts ^a
100%	3.23	0.35 ± 0.12 (3)	2.39	2.24 ± 0.12 (3)	3.47	0.60 ± 0.58 (3)
60%	2.15	0.63 ± 0.10 (3)	1.44	2.58 ± 0.16 (3)	2.75	0.82 ± 0.72 (3)
40%	1.66	1.06 ± 0.12 (3)	1.17	3.67 ± 0.20 (3)	2.56	1.27 ± 0.97 (3)
20%	0.95	1.04 ± 0.21 (3)	0.65	2.75 ± 1.27 (3)	1.07	1.06 ± 0.75 (3)
10%	0.67	1.28 ± 0.44 (3)	0.45	3.85 ± 0.29 (3)	0.61	1.10 ± 0.78 (3)
5%	0.76	1.10 ± 0.10 (3)	0.29	2.77 ± 0.76 (3)	b	Not Tested
Control	0.14 ^b	1.00 ± 0.84 (3)	0.20 ^b	3.20 ± 0.16 (3)	0.22 ^b	1.06 ± 0.81 (3)

^a Cells × 10⁶/mL ± 1.0 S.D. (n = sample size).

^b background levels.

The ANOVA of the mean cell counts for Batch 1 fog oil showed significant interaction effects and effects over time, but treatment (fog oil concentration) effects were not significant (Table 49). At 96 hr, a posteriori multiple pairwise comparisons showed no significant differences between any pairs of means using Scheffe's critical value (Table 50). The less conservative tests (without Scheffe's correction) showed that the two highest exposure treatments (100% and 60% treatments) were significantly different from all other treatments, but were not different from each other ($p < 0.1$). The degree of growth inhibition in the high exposure was 65% compared to the controls.

TABLE 49. ANALYSIS OF VARIANCE (ANOVA) OF MEAN CELL COUNT DATA FROM THE ALGAL TOXICITY TEST WITH BATCH 2 FOG OIL

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Prob.
Mean	4923122.84272	1	4923122.84272	136.61	0.0000
Cell Counts	462266.30184	6	77044.38364	2.14	0.1182
Error	468482.22032	13	36037.09387		
Time	11965023.79693	4	2991255.94923	102.18	0.0000
Interaction	1383690.53783	24	57653.77241	1.97	0.0208
Error	1522338.13714	52	29275.73341		

TABLE 50. F-VALUES FOR COMPARISONS BETWEEN ALL POSSIBLE PAIRS OF MEANS AT 0, 24, 48, 72, AND 96 HOURS FOR BATCH 1 FOG OIL

Treatment Contrast	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
100% - 60%	0.00	0.00	0.00	0.00	3.38
100% - 40%	0.00	0.00	0.00	0.02	21.10
100% - 20%	0.00	0.00	0.00	0.01	20.39
100% - 10%	0.00	0.00	0.02	0.21	36.28
100% - 5%	0.00	0.00	0.00	0.59	16.28
100% - Control	0.00	0.00	0.11	0.79	17.90
60% - 40%	0.00	0.00	0.00	0.02	7.60
60% - 20%	0.00	0.00	0.00	0.00	7.17
60% - 10%	0.00	0.00	0.03	0.19	17.52
60% - 5%	0.00	0.00	0.00	0.56	5.72
60% - Control	0.00	0.00	0.13	0.74	5.73
40% - 20%	0.00	0.00	0.00	0.00	0.01
40% - 10%	0.00	0.00	0.01	0.10	2.04
40% - 5%	0.00	0.00	0.00	0.41	0.01
40% - Control	0.00	0.00	0.09	0.55	0.13
20% - 10%	0.00	0.00	0.01	0.14	2.27
20% - 5%	0.00	0.00	0.00	0.47	0.00
20% - Control	0.00	0.00	0.08	0.63	0.08
10% - 5%	0.00	0.00	0.01	0.13	1.83
10% - Control	0.00	0.00	0.03	0.18	3.21
5% - Control	0.00	0.00	0.08	0.00	0.06

The ANOVA for the Batch 2 test was significant for treatment, effects over time and interactions (Table 51). Tests with Scheffe's correction showed no significant differences between any treatment means (Table 52). The less conservative tests showed a number of differences at $P > 0.05$. At 48 and 72 hr, the control response was different than the 100%, 60% and 5% treatments. At 96 hr, the control treatment was different than the 100%, 40% and 10% treatments. The apparent degree of inhibition was approximately 40% in the 100% (2.24 mg/L) treatment. Other tests among treatments at 96 hr were significant. These include the following: the 60% treatment was different than the 40% and 10% treatments, the 40% treatment was different than the 20% and 5% treatments, the 20% and 10% treatments were different, and the 10% and 5% treatments were different. These random fluctuations indicate the degree of variability in response observed at oil concentrations between 0.29 and 1.44 mg/L.

TABLE 51. ANALYSIS OF VARIANCE (ANOVA) OF MEAN CELL COUNT DATA FROM THE ALGAL TOXICITY TEST WITH BATCH 2 FOG OIL

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Prob.
Mean	173255083.72342	1	173255083.72342	1032.17	0.0000
Cell Counts	5697295.54964	6	949549.25827	5.66	0.0044
Error	2182117.23051	13	167855.17158		
Time	109120756.46799	4	27280189.11700	299.77	0.0000
Interaction	6640780.00750	24	276699.16698	3.04	0.0004
Error	4732187.99545	52	91003.61530		

TABLE 52. F-VALUES FOR COMPARISONS BETWEEN ALL POSSIBLE PAIRS OF MEANS AT 0, 24, 48, 72, AND 96 HOURS FOR BATCH 2 FOG OIL

Treatment Contrast	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
100% - 60%	0.00	0.26	0.77	0.73	1.06
100% - 40%	0.00	0.12	1.49	5.21	18.26
100% - 20%	0.00	0.11	1.46	8.40	23.36
100% - 10%	0.00	0.02	2.74	13.38	23.36
100% - 5%	0.00	0.01	0.05	1.29	2.20
100% - Control	0.00	0.01	9.43	14.19	8.32
60% - 40%	0.00	0.03	0.11	2.04	10.51
60% - 20%	0.00	0.03	0.11	4.18	0.26
60% - 10%	0.00	0.14	0.60	7.86	14.45
60% - 5%	0.00	0.29	0.31	0.14	0.31
60% - Control	0.00	0.15	4.80	8.49	3.43
40% - 20%	0.00	0.00	0.00	0.38	7.47
40% - 10%	0.00	0.04	0.19	1.89	0.31
40% - 5%	0.00	0.15	0.74	0.82	5.47
40% - Control	0.00	0.05	3.43	2.21	1.93
20% - 10%	0.00	0.03	0.20	0.58	10.84
20% - 5%	0.00	0.14	0.73	2.12	0.01
20% - Control	0.00	0.04	3.48	0.76	1.81
10% - 5%	0.00	0.04	1.57	4.56	8.07
10% - Control	0.00	0.00	2.00	0.01	3.80
5% - Control	0.00	0.03	6.35	4.99	1.20

TABLE 53. ANALYSIS OF VARIANCE (ANOVA) OF MEAN CELL COUNT DATA FROM THE ALGAL TOXICITY TEST WITH BATCH 3 FOG OIL

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Prob.
Mean	6848585.38618	1	6848585.38618	282.29	0.0000
Cell Counts	143971.73991	5	28794.34798	1.19	0.3758
Error	266866.15177	11	24260.55925		
Time	11647756.33159	4	2911939.08290	125.17	0.0000
Interaction	707768.58784	20	35388.42939	1.52	0.1222
Error	1023608.67128	44	23263.83344		

The ANOVA of the Batch 3 test was not significant for treatment or interaction effects between time and treatment effects (Table 53). No further statistical comparisons of treatment means were conducted. Inspection of the data suggests 40% inhibition of growth at 96 hr between the 3.47 mg/L treatment and the control treatment.

An algal toxicity test was performed with vaporized Batch 3 fog oil (FO-4). The ANOVA (Table 54) was not significant for treatment or interaction effects and no further testing was conducted. Inspection of the data (Table 55) suggests no indication of a dose dependent response.

As a result of the Phase II Screening toxicity tests, it was concluded that there was very little if any toxicity associated with fog oil. Moreover, there was no firm indication that one batch was more toxic than another. Batch 3 was selected for vaporization and photolysis studies because it was the most recently manufactured batch of fog oil. By outward appearances, it was the least oxidized batch of oil. Acute toxicity tests were performed with bulk and vaporized Batch 3 fog oil with all four invertebrate species. P. promelas were also tested at a screening level with vaporized fog oil. H. azteca was used in the photolysis experiments.

The results of the D. magna tests with bulk and vaporized fog oil closely paralleled the Phase I tests. The principle response was the phenomenon of floaters caused by adherence of fog oil to the organisms (Table 56). The stock solutions generated for these tests contained considerably more fog oil than prior tests. This was caused by a bend in the shaft of the stirring propeller which created greater mixing energies during the mix. As indicated in the Phase I tests, it was not possible to separate the toxic properties of the oil from the physical effects. Comparisons can be made where exposure concentrations overlap between the bulk and the vaporized fog oil. Initial exposure concentrations of

TABLE 54. ANALYSIS OF VARIANCE (ANOVA) OF MEAN CELL COUNT DATA FROM THE ALGAL TOXICITY TEST WITH VAPORIZED BATCH 3 FOG OIL

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Prob.
Mean	8566518.16565	1	8566518.16565	380.34	0.0000
Cell Counts	194390.07682	5	38878.01536	1.73	0.2032
Error	270281.48659	12	22523.45722		
Time	11932427.90291	4	2983106.97573	179.76	0.0000
Interaction	442194.85531	20	22109.74277	1.33	0.2055
Error	796553.32236	48	16594.86088		

vaporized fog oil were lethal at 8.96 mg/L after 48 hr (Table 57). The stock solution of the bulk fog oil (30.6 mg/L) was 80% lethal at 48 hr suggesting that vaporization had increased the toxicity of the fog oil. When it became apparent that the stock solutions had higher than expected levels of fog oil, additional exposure beakers were set up at nominal concentrations of 1.0% and 0.1% of the stock solutions. These treatments provide an estimate of the amount of oil necessary to cause the floating response. Base on the results from the two tests, the concentration of fog oil required to produce floaters is quite low, on the order of ~0.10 to 0.30 mg/L total oil.

Biological endpoints monitored in the acute toxicity tests with P. dissimilis included mortality and the number of tubes constructed by the

TABLE 55. RESPONSES OF SELENASTRUM CAPRICORNUTUM TO VAPORIZED BATCH 3 FOG (96-HR DATA)

Treatment	Total Oil (mg/L)	Mean (\pm 1.0 S.D.) Cell Counts ($\times 10^6$ /mL)
100%	2.38 \pm 0.07	0.83 \pm 0.16
60%	1.48 \pm 0.06	1.02 \pm 0.60
40%	1.10 \pm 0.09	0.95 \pm 0.36
20%	0.76 \pm 0.07	0.97 \pm 0.17
10%	0.68 \pm 0.00	1.35 \pm 0.04
0%	0.18 \pm 0.02	0.84 \pm 0.10

TABLE 56. OBSERVATIONS (%) OF DAPHNIA MAGNA EXPOSED TO OMM OF BATCH 3 BULK FOG OIL

Treatment	Total Oil (mg/L)	No. of Organism	24 hr				48 hr			
			Floaters		Free		Floaters		Free	
			Alive (%)	Dead (%)	Swimming (%)	Dead (%)	Alive (%)	Dead (%)	Swimming (%)	Dead (%)
100%	30.63 ± 0.65	20	50	45	0 ^a	80 ^a	20	80 ^a	0 ^a	0
60%	16.99 ± 1.12	20	65	35	0	65	35	65	0	0
40%	12.43 ± 0.47	19	42	58	0	89	11	89	0	0
20%	6.26 ± 0.12	19	89	11	0	50	50	50	0	0
10%	3.60 ± 0.13	20	90	10	0	45	55	45	10	10
1%	(0.30) ^b	20	85	0	15	35 ^a	55	35	10	10
0.1%	(0.03) ^b	20	80	5	15	0	55	0	100	100
Control	0.16 (Bkg)	20	0	0	100	0	0	0	0	0

^a One dead organism on bottom of beaker.^b Nominal concentration based on dilution of stock solution.TABLE 57. OBSERVATIONS OF DAPHNIA MAGNA EXPOSED TO OMM OF BATCH 3 VAPORIZED FOG OIL

Treatment	Total Oil (mg/L)	24 hr				48 hr			
		Floaters		Free		Floaters		Free	
		Alive (%)	Dead (%)	Swimming (%)	Dead (%)	Alive (%)	Dead (%)	Swimming (%)	Dead (%)
100%	71.95 ± 3.27	80	20	0	100	0	100	0	0
60%	46.17 ± 4.59	100	0	0	100	0	100	0	0
40%	33.59 ± 3.00	100	0	0	100	0	100	0	0
20%	17.16 ± 0.66	100	0	0	100	0	100	0	0
10%	8.96 ± 0.16	100	0	0	100	0	100 ^b	0	0
1%	(0.72) ^a	94	0	0	17	25 ^c	65	0	0
0.1%	(0.072) ^a	100	0	0	100	0	0	100	100
Control	0.08 ± 0.08	0	0	0	0	0	0	0	0

^a Nominal concentrations.^b One handling mortality on 18 total organisms for this treatment.^c Two free swimming organisms.

larvae (Table 58). Mortality was a difficult parameter to measure because in many instances the larvae would not move once they were located in the test beakers and removed for quantification. Mortality at 48 hr in the vaporized and bulk fog oil tests didn't exceed 50% in spite of the fact that the stock solutions used for these tests were considerably more concentrated than those used in the Phase II screening tests. In contrast with the D. magna tests, bulk fog oil appeared to be more toxic than vaporized fog oil. Decreases in tube building in both tests were indicative of adverse effects of the fog oil but were not statistically analyzed. The number of tubes constructed as well as the length of the tubes decreased with increasing fog oil concentration. Mortality and tube construction were noticeably effected at approximately 12 to 17 mg/L total oil. It is not known what effect, if any, the presence of substrate had on the toxicity of the oil.

In the course of performing the planned Phase II tests and the photolysis tests, several tests were performed with bulk and vaporized batch 3 fog oil and H. azteca. Screening tests with Batch 3 bulk fog oil indicated that 14.8 mg/L total oil was not acutely toxic to H. azteca (Table 59). A screening test with vaporized fog oil indicated that vaporization had little effect on toxicity. A Phase II test with vaporized fog oil had only five percent mortality at 48 hr in the highest concentration tested

TABLE 58. EXPOSURE OF PARATANYTARSUS DISSIMILUS TO OWM OF BULK AND VAPORIZED BATCH 3 FOG OIL

Treatment	Total Oil (mg/L)	Number	Mortality (%)	No. of Tubes
-----Bulk-----				
100%	30.63 ± 0.65	20	25	0
60%	16.99 ± 1.12	19	26	6
40%	12.43 ± 0.47	20	5	14
20%	6.26 ± 0.12	19	10	18
10%	3.60 ± 0.13	19	20	13
Control	<0.16	20	0	25
-----Vaporized-----				
100%	71.95 ± 3.27	20	35	1
60%	46.17 ± 4.59	20	30	4
40%	33.59 ± 3.00	19	11	2
20%	17.16 ± 0.66	20	5	12
10%	8.96 ± 0.16	20	0	24
Control	0.08 ± 0.08	20	0	31

TABLE 59. SUMMARY OF *HYALLELA AZTECA* MORTALITY (%) IN SCREENING TOXICITY TEST WITH PHOTOLYZED BATCHES OF BULK AND VAPORIZED FOG OIL^a

	24 hr	48 hr
-----Bulk Oil-----		
100% stock (14.8 mg/L) ^b	0	6
20% stock	0	0
Control	0	6
-----Photolyzed Bulk Oil-----		
100% stock (66.3 mg/L) ^b	25	100
20% stock	0	0
Control	6	6
-----Vaporized Oil-----		
100% stock (64.3 mg/L) ^b	13	25
20% stock	0	0
Control	0	0
-----Photolyzed Vaporized Oil-----		
100% stock (188 mg/L) ^b	100	100
20% stock	25	63
Control	6	6

^a Sixteen organisms per treatment.

^b By IR analysis.

(5.58 mg/L total oil). As in other tests with bulk and vaporized fog oil, the exposure solutions in these tests had oil slicks floating on the surface of the exposure solutions at 24 hr. Because total mortality didn't exceed 50%, EC50 values were not calculated.

Photolysis of Fog Oil

Four screening-level toxicity tests were performed with *H. azteca* as the test species. Four treatments of Batch 3 SGF No. 2 Fog Oil were used in tests: bulk fog oil (untreated), vaporized fog oil (FO-4), and 24-hr photolyzed treatments of both. The photolyzed and non-photolyzed oil were tested ~5 to 6 days after photolysis.

The mortality data from all four tests are summarized in Table 59. It is readily apparent that toxicity of the bulk fog oil had not noticeably

TABLE 60. TOXICITY TESTING RESULTS OF HYALLELA AZTECA AND BULK FOG OIL, 7-DAY PHOTOLYZED BULK FOG OIL, VAPORIZED FOG OIL, AND PHOTOLYZED-VAPORIZED FOG OIL

Treatment	Total Oil ^a (mg/L)	Mortality (% , n = 15)	
		24 hr	48 hr
-----Bulk-----			
100%	2.02	0	0
20%	(0.40)	0	0
-----Photolyzed Bulk-----			
100%	18.92	40	93 ^b
60%	(11.1)	0	38 ^b
40%	(7.4)	7	7
20%	(3.7)	0	7
10%	(1.9)	0	0
Control	0	0	0
-----Vaporized-----			
100%	7.81	0	0
20%	(1.6)	0	0
-----Photolyzed-Vaporized-----			
100%	20.81	60	93
60%	(12.5)	20	60
40%	(8.3)	7	7
20%	(4.2)	0	0
10%	(2.1)	0	7
Control	0	0	0

^a By UV adsorbance with isooctane extracts, nominal concentrations are in parentheses.

^b n = 13.

TABLE 61. RETESTING OF 100% EXPOSURE SOLUTIONS OF 7-DAY PHOTOLYZED FOG OIL

Treatment	Total Oil ^a (mg/L)	Mortality (% , n = 15)	
		24 hr	48 hr
Photolyzed Bulk	12.7	27	93 ^b
Photolyzed-Vaporized	16.7	13	87 ^b
Control	0	0	0

^a By UV adsorbance, isooctane extracts.

^b All living organisms were severely incapacitated.

changed. In spite of higher oil concentrations than observed with the standard slow mix, the organisms did not become mired in the surface oil slicks. After 48 hr, only 1 of 16 organisms exposed to the 100% exposure solution had died. There were no mortalities in the 20% or control treatments. The mortality pattern in the test with vaporized fog oil, however, indicated a higher level of mortality than was observed in the Phase II test with the standard slow mix. This may be explained by the higher level of fog oil in the exposure beakers.

Toxicity tests with H. azteca were performed with batches of bulk and vaporized (FO-4) SGF No. 2 fog oil that had been photolyzed for 7 days. The mixing regimen was modified because the method used for the screening tests with 24-hr photolyzed fog oil was considered to be too variable for standardization (see section 2.6.5). Only 100% and 20% exposure solutions of unphotolyzed bulk and vaporized fog oils were prepared from these mixes. At the end of the test, the 100% photolyzed solutions were retested. This involved separating the aqueous phase from the photolyzed oil residues floating on the surface by siphoning under low vacuum. This was done to each of three replicate beakers for the 100% exposure solutions of the photolyzed bulk and vaporized fog oil. The non-photolyzed bulk and vaporized oils were not tested because they did not show any toxicity in the initial test.

As noted in the first tests with 24-hr photolyzed oil, a major effect of photolysis on fog oil is the apparent increase in the ability of the oil to mix with water. The 100% stock solutions of photolyzed oils were approximately 2 to 20 times higher in total oil than the unphotolyzed oils (Table 60). A direct comparison of the 10% solutions of photolyzed bulk oils with the 100% solutions of the unphotolyzed bulk fog oils can be made on the basis of total oil concentrations. No mortality was noted in any of these treatments. The 100% and 60% solutions of both photolyzed oils were clearly toxic. The second series of tests with the siphoned fraction clearly showed that the toxic constituents remained in solution and did not separate out of solution (Table 61). The observed toxicity may be attributed to the higher concentrations of oil in water or an increase in the toxic character of the constituents of the photolyzed fog oil. In either case, photolysis increased the toxicity of SGF No. 2 fog oil. Moreover, the observed toxicity is most likely attributed to increased solubility of fog oil hydrocarbons.

4.0 DISCUSSION

This chapter integrates those aspects of aerosol generation, chemistry and toxicity testing necessary to evaluate the environmental hazards of RP-BR and WP-F and fog oil obscurants to aquatic organisms. The effect acidification has on the toxicity of phosphorus-based obscurants is also discussed. Toxicity testing with fog oil indicated that the obscurants were relatively non-toxic and that fouling caused by direct contact with oil is the primary concern. Photolysis was shown to increase the presence of soluble components in the oil and the resulting toxicity. Issues that need to be considered when attempting to extrapolate these laboratory data to field exposure situations are discussed at the end of the chapter.

4.1 PHOSPHORUS-BASED OBSCURANTS

This section discusses aerosol generation, chemistry, and toxicity of RP-BR and WP-F. Phase I tests determined the effects of aging and neutralization on the chemistry and toxicity of these materials in water. The Phase II tests provided definitive information on how total P and pH affect the toxicity of oil. Results of the Phase I tests are compared with published data on the tolerance of aquatic organisms to toxicity and acidification.

4.1.1. Aerosol Generation

Suitable methods for dosing water with RP-BR and WP-F smoke were developed in Phase I of the project. Initially, both bubbling and settling methods were tested; both were found to influence the percentage of phosphate and phosphite in the stock solutions. Settling of smoke on water was judged to be more representative of field conditions and, therefore, all subsequent dosing of exposure water used settling in either 54- or 760-L carboys.

The lack of agreement between mean smoke concentration and total P in the stock solutions in the 54-L carboy dosing system remains unresolved. Phosphorus combustion products are hygroscopic and retain an unknown amount of H_2O after desiccation. Filter pad weights taken during smoke generation would overestimate the P content of the smoke. Ash from the matrix materials may also inflate the measured concentrations of smoke, though this source is considered to be negligible. Incomplete settling of smoke prior to rinsing may have contributed to the lower-than-expected concentrations of P in the stock solutions, but a 1.5-L filter grab sample taken from a 54-L carboy one hour after settling was negative. Therefore, it is reasonable to assume that settling was at least 99% complete at the time stock solutions were removed from the carboys. It is also possible that handling the carboys while rinsing could have resuspended some of the aerosol. Based on the reported solubilities of the various oxyphosphoric acids,

incomplete dissolution and adherence to the sides of the carboys is unlikely.

It is possible that the geometry of the carboys and the temperature differentials between the smoke and the surfaces of the carboy, and between the smoke and the water in the carboy created convection currents that minimized the settling and sorption of smoke particles in the system. This does not account for the low recovery of mean smoke concentrations, however. Resolution of this discrepancy would require extensive modeling of aerosol dynamics within the carboys and is beyond the scope of the project. Although the relationship between total P in the stock solutions and the mean smoke concentration in the 760-L carboys agreed with what was expected (i.e., the concentration of P in the stock solution exceeded the mean smoke concentration), uncertainties in the smaller carboy system cast doubt on the validity of these numbers. The discrepancy may be, in part, attributed to unremovable water bound to the smoke particles by hygroscopic forces; analysis for total P on the filters was not included in the analysis regimen.

4.1.2 Aqueous Chemistry

Chemical changes in the smoke before and after entry into water may influence the speciation and degree of acidification of the receiving water. These changes, in turn, influence the resulting toxicity. Combustion of RP-BR or WP-F obscuring agents yields phosphorus oxides ranging up to the pentoxide (P_4O_{10}) if combustion is complete. Lower oxides such as the trioxide (P_4O_6) are enhanced when oxygen is limited. Upon contact with water, the oxides form acidic species, including linear polyphosphates and small amounts of cyclic metaphosphates. The polymers will eventually break down to form phosphoric acid, but reaction rates vary and total hydrolysis is slow. The trioxide also provides a potential pathway for formation of the highly toxic phosphine gas (PH_3). This gas has low water solubility and presents a minimal potential for aquatic toxicity.

Stock solutions were characterized routinely for total P and for percentages as phosphate and phosphite to give an indication of the reproducibility of the dosing methodology. Selected samples were further speciated for polyphosphate distribution. Analysis for hypophosphite generally showed levels near or below detection limits, which would account for no more than 0.1% of the total phosphorus in solution. Phosphite levels, although low, were more easily measured. Although the initial bubbled RP-BR smoke test (RP-1) resulted in elevated (2.5%) phosphite levels, all subsequent levels were lower, usually running well below 1% for RP-BR burns and about 0.1% for WP-F burns. Phosphate levels ranged from 8% to 20% in fresh solutions from 54-L carboy dosings. The large (760-L) fusion carboy containers used to generate smoke-dosed waters for fish studies ranged from 15% to 40% initial PO_4 -P. Part of the variation may have been caused by the change in settling time allowed by the dosing methods.

Although the toxicity tests were relatively short (≤ 96 hr), aging of the solutions over a 26-day period prior to exposure with Daphnia magna did point out the concentration/pH dependence on hydrolysis rate and solubility of P species in well water. The critical level at which precipitation occurred was about pH 6; neutralization of exposure solutions tended to result in more rapid initial precipitation, followed by a long-term period of slow precipitation. Very hard waters tended to precipitate even without neutralization; the inherent alkalinity of the water caused partial neutralization of the acidic species. Although the precipitated material was not fully characterized, it could include components such as amorphous particles of alkaline earths and/or mixed salts of tripolyphosphates and pentapolyphosphates (Van Wazer 1958).

Speciation studies done after the program was well under way indicated that neutralization of fresh stock solutions apparently caused a change in the hydrolysis pathway or in rates of hydrolysis steps. Aged neutralized solutions tended to show elevated levels of cyclic trimetaphosphate relative to unneutralized solutions. The observed phosphate levels gave little indication of this change in hydrolysis. A rate-determining step in overall hydrolysis may involve the 3P and/or 3mP species.

4.1.3 Aquatic Toxicity

The acute toxicity of RP-BR and WP-F smoke to nine different aquatic organisms was determined. Tests with D. magna during Phase I of the project were extended from 48 to 96 hr. These extended tests revealed a bimodal response in smoke-dosed well water. The initial response was related to acidification of the exposure water and was readily apparent within 24 to 48 hr of exposure. At 72 and 96 hr, a second phase of mortality was observed in the treatment containing 30% to 50% stock solution. This phase of mortality may have been related to the formation of precipitated material that accumulated in the gastrointestinal (GI) tract of the exposed daphnia. The extent to which materials would accumulate under field situations is unclear. In laboratory situations, the confines of an exposure beaker increase the interaction of the test organisms with the precipitated material. In field situations, the precipitated material is no longer available after it settles into the sediment. The significance of ingestion of precipitated material depends on whether the cause of death was due to functional blockage of the GI tract, chemical toxicity, or a combination of the two factors. This secondary mode of toxicity may have little ecological significance.

The aging tests with D. magna in well water showed that exposure solutions below a pH of 5 changed little in toxicity over time. The secondary toxicity related to precipitated material disappeared at 26 to 28 days. This observation corroborates our conclusion that the precipitated material is of little ecological significance. The potential for different aquatic systems to recover from acidification defines the long-term hazards associated with these materials. Acutely toxic effects would continue until

natural or artificial processes elevate the pH. The neutralization tests with reconstituted water indicated presence of toxic smoke constituent(s) that acted independently of pH. This phenomenon was apparent in Phase I tests with D. magna and in Phase II tests with Selenastrum capricornutum. Toxicity was only observed in the most concentrated solutions tested. A potential hazard to aquatic systems exists when sufficient amounts of smoke enter highly buffered water. The high buffering capacity negates pH-related toxicity but allows levels of unidentified toxic constituents to accumulate.

Although red phosphorus is insoluble in water, white phosphorus shows measurable solubility at about 3 mg/L (Berkowitz et al. 1981) and, therefore, could present a significant mode of toxicity to freshwater organisms. The concentration of dissolved white phosphorus in several stock solutions was analyzed by solvent extraction and ICP analysis. In most cases, the concentrations were near or below detection limits and appeared to represent no more than 0.01% of the total P in solution. While this level would be toxic to the more sensitive fish in a 20 mg/L total P solution, the major source of toxicity remains the pH of the resultant solution. Toxicity in neutralized exposure solutions was observed only in neutralized solutions in excess of 70 mg/L total P.

Since WP-F toxicity to fish was reported to be on the order of 2 $\mu\text{g/L}$ (Sullivan et al. 1979; Berkowitz et al. 1981), this level could be significant in a 20 mg/L total P solution. The EPA recommended limit for total P in marine or estuarine water is 0.1 $\mu\text{g/L}$ (Environmental Protection Agency 1976). The recommended discharge standard is 0.01 $\mu\text{g/L}$ (Lai 1979). Actual analysis for $\mu\text{g/L}$ levels of WP-F would require the minimum of a GC procedure, which was not available for this study. Since the acid effect on the smoke-dosed water was the major source of toxicity in this study, neutralization of solutions would be the only method that could indicate any increased toxicity caused by the possible presence of dissolved elemental P or specific combustion products.

Acute toxicity of RP-BR and WP-F combustion products in water was directly related to the amount of acid generated from these materials. The extent to which a body of water will be acidified is a function of the buffering capacity of that water, the amount of smoke that enters the water, and the composition of the smoke. The pH of most freshwater systems is moderated by the amount of carbonate present in the system. The carbonate buffering system affords the highest degree of buffering about the pKa values of 6.43 at 15°C (i.e., a pH range of 5.4 to 7.4). The addition of oxy-phosphoric acids to the system also introduces a second major buffering system, that formed by the presence of di- and mono-basic phosphates. The respective pKa values for these phosphoric acid species are 7.21 and 2.12. Collectively, the carbonate and phosphoric acid buffering systems offer little buffering capacity at pH values between 3.0 and 5.4. The larger polyphosphates may also contribute to the buffering capacity of the receiving water. Collectively, these anions contribute to the pH of the

receiving water. The variable composition of the smoke and our lack of understanding of how the different phosphate species contribute to the buffering capacity of the water contributes to the variability between pH and total P observed in the stock solutions used for toxicity testing.

The lack of buffering capacity between pH values of 3.0 and 5.4 affected the test methodology. This region of low buffering capacity overlaps the range of pH values that were acutely toxic. When concentrated stock solutions were used, small errors in the preparation of exposure solutions caused significant changes in the resulting pH of the exposure solutions. This made it difficult to obtain a range of pH levels in which there would be partial mortality. Moreover, a narrow range of pH values bracketed 100% mortality and 100% survival in the tests with fish and invertebrates. In tests with no partial mortality, the confidence intervals about the LC50 cannot be estimated. These LC50 values agree with published accounts (discussed later) of the toxicity associated with acidification.

The toxicity tests with *S. capricornutum* need to be evaluated from the perspective of pH because algal media has a lower buffering capacity than most surface bodies of water. Use of total P as an indication of toxicity could inadvertently bias the assessment if the buffering potential of the media is not considered. The results of the algal toxicity test with WP-F were highly variable within treatments. This variation may be attributed to the inoculation level of 10,000 cells/mL and to the use of three replicate flasks for each treatment. A higher inoculum level and additional flasks per treatment could have reduced the variability. The trends in the WP-F test were not significant but paralleled the trends in the RP-BR test, which were significant.

The toxicity tests indicated that algal growth was inhibited at pH levels ≤ 6.0 and stimulated at pH levels >6.0 . Suppression of growth was also observed in the algal stimulation tests at pH ~ 5.5 . Miller, Greene and Shiroyama (1978) reported appreciable growth in 14-day maximum yield tests at an initial pH of 5.0. Growth in flasks with an initial pH of 5 sustained approximately 90% of the growth in flasks at an initial pH of 6 to 9 over 14 days. Growth at 96 hr was not reported. It is reasonable to assume that the pH levels would change over the 14-day period of the test and that growth at 4 days could have indicated a significantly different relationship to pH. Photosynthesis increases pH levels by removing CO_2 and shifting the bicarbonate equilibrium during the light cycle. Consequently, the data of Miller, Greene and Shiroyama (1978) and the data from our toxicity tests cannot be compared directly.

Stimulation of algal growth by phosphorus smoke was demonstrated at low smoke concentrations that did not acidify the media. The final concentration of PO_4 was saturating in both neutralized and non-neutralized treatments. Because of the low buffering capacity of the algal media, the amounts of total P compared to pH are relatively small with respect to other types of water dosed in this project. Most green algae require

between 0.02 and 0.50 mg $\text{PO}_4^{3-}\text{P/L}$ for optimal growth (Wetzel 1975). Blue-green algae require higher concentrations of PO_4^{3-} , up to 3.00 mg $\text{PO}_4^{3-}\text{P/L}$. It is apparent that small amounts of smoke entering a body of water could significantly affect algal growth before changes in pH would become perceptible. The potential for continued stimulation of algal growth depends on the rate of hydrolysis of the longer chain polyphosphates and the biogeochemical cycling of P and other nutrients in the system.

The hazards presented by the introduction of the combustion products of phosphorus-based obscourants into aquatic systems are similar to the hazards presented by acid rain. It has been recommended that a criterion for the degree of acidification capable of adversely affecting aquatic habitats be lowered to a pH range from 5.0 to 6.0 (Henlrey 1984). This recommendation is founded upon a growing body of data from field studies of lakes influenced by acid precipitation. All major categories of freshwater life, including fish, invertebrates, and algal and microbial communities, are affected at pH levels below 6.0.

Fish

Numerous studies have been performed to define the sensitivity of fish to acid-induced stress. Ellgaard and Gilmore (1984) reported the 96-hr LC50 values of four reagent grade acids to bluegill sunfish. The 96-hr LC50 for phosphoric acid fell between a pH of 3.25 and 3.00. The testing methodology involved exposing bluegill sunfish to decreasing increments of acid for 96-hr intervals. After 96 hr of exposure to a specified pH level, the pH was lowered to the next level. The pH levels tested were 7.5, 5.0, 4.5, 4.0, 3.5, 3.25 and 3.00. Thirteen percent mortality was observed at 24 hr at a pH of 3.25 and did not change over the remaining 96 hr duration of the test. After adjusting the pH to 3.00, mortality increased to 100% in the first 24 hr of exposure.

The physiological and toxicological implications of acid-induced stress in fish was recently review by Fromm (1980). Severe acid stress causes an accumulation of mucus on the gills that interferes with normal gas exchange resulting in hypoxia and, ultimately, death. Sub-lethal acid stress results in a decrease in blood pH and disruption of homeostatic mechanisms. Effluxes of H^+ and CO_2 are reduced and Na may be lost across gill membranes. Higher, less debilitating pH levels (5.0 to 6.5) may have subtle adverse affects on reproduction, i.e., depressed oocyte production, calcium regulation.

In the Experimental Lakes Area of Canada, long-term studies involving the artificial acidification of Lake 223 have shown a trend toward a reduction in the recruitment of lake trout (Salvelinus namaycush) and white sucker (Catostomas commersoni) at pH levels between 5.0 and 5.4 (Mills 1984). Recruitment failure was noted in fathead minnow at pH levels of 5.8 two years before failure of the lake trout and white sucker populations.

Invertebrates

Declines in the diversity of Chydorid cladocerans in New England Lakes has been attributed to increased acidification caused by atmospheric deposition (Brakke, Davis and Kenlan 1984). Sedimentary remains of the exoskeletons of these macroinvertebrates were recovered from sediment cores and examined as a function of age to evaluate changes in the community structure. The data indicated that many species of chydorids tolerated pH values as low as 4.4. Species diversity increased in higher elevation lakes, presumably because of the lower buffering capacity of these oligotrophic lakes.

Aquatic Insects

Aquatic insects are relatively tolerant to acidification. Bell and Nebeker (1969) investigated the acute toxicity of acid on 10 species of larval aquatic insects representing four distinct orders: dragonfly (Odonata), stonefly (Plecoptera), caddisfly (Tricoptera) and mayfly (Ephemeroptera). The most insensitive species tested were two species of caddisflies (Brachycentrus americanus and Hydropsyche betteni), which had 96-hr median tolerance limits (TLM) for pH values of 1.5 and 3.15. The most sensitive species tested were the stonefly Pteronarcys dorsata and the mayfly Ephemerella subvaria, which had mean 96-hr TLM for pH values of 4.25 and 4.65, respectively. Long-term chronic exposures of the same species for 30 days increased the TLM of the pH. Values that caused 50% mortality after 30-day exposure to acid were 0.23 to 1.17 pH units greater than the pH values associated with 96-hr TLM values (Bell 1971). The relative sensitivity of the species tested in the two studies was similar. The mayfly (E. subvaria) was the most sensitive species tested; it had a 30-day TLM of 5.38. Adult emergence was a more sensitive indicator of acid-induced stress than the 30-day TLM. The stonefly Isogenus frontalis had a 50% success rate of emergence at a pH of 6.6. The next two most sensitive species were P. dorsata and E. subvaria, the species that were most sensitive to acute pH levels.

4.2 SGF NO. 2 FOG OIL

The relative hazards of bulk, vaporized and photolyzed batches of bulk and vaporized fog oil are discussed with respect to the effects these processes had on the aqueous chemistry and aquatic toxicity of the fog oil. Our results are compared to related data on other organic liquids.

4.2.1 Fog Oil Vapor Generation

At the start of the project, both settling and bubbling of fog oil vapor were considered as methods for dosing water. The option of bubbling fog oil vapor through water was not investigated because the initial trial burns with RP-BR suggested the method was inefficient and did not represent natural processes. Scrubbing the vapor was perhaps the most efficient

method of collecting fog oil vapor, but the resulting emulsion did not represent a medium that one would realistically expect to find in aquatic systems. Collecting fog oil vapor on glass wool filters was time consuming and inefficient. The method of settling of fog oil vapor was selected because it most closely simulated field conditions by allowing sufficient time for the aerosol to age.

The initial generation of fog oil vapor was conducted with a 100% N₂ atmosphere. In remaining vaporizations, the N₂ was augmented with compressed air to more closely approximate the conditions under which fog oil vapor is generated in the field with the M3A3 generator. In the field, fog oil is vaporized on the exhaust manifold of the generator and is then transported from the generator by entrainment in the exhaust of the gasoline engine. Oxidation of fog oil constituents would not occur in a pure N₂ environment. Oxidized fog oil constituents would be expected to be more soluble in aqueous solution and, consequently, potentially more toxic to aquatic organisms. Although vapor generation in the presence of 6% air approximates the stoichiometric amount of O₂ in a finely tuned gasoline engine, these conditions fall short of duplicating the conditions present in the M3A3 generator because constituents normally found in gasoline motor exhaust were absent in the generation system used on the project. Although the presence of free radicals was not determined in the first vaporization experiment (FO-1), the vaporized fog oil generated in that test was not as dark as the Batch 3 fog oil vaporized with 6% air (FO-4) in Phase II tests. The method used in the project provided the most representative generation of fog oil vapor for aquatic toxicity testing short of setting up a M3A3 generator in the lab.

4.2.2 Fog Oil Chemistry

Analysis of the three batches of fog oil revealed few differences among them. The presence of the unresolved envelope in gas chromatograms was consistent with analyses performed by Katz et al. (1980). The relative coloration of the oil was indicative of its age and extent of oxidation. Batch 1 was the oldest and, like Batch 2, arrived in a previously opened barrel. Batch 1 was distinctly darker than Batch 2. Batch 3 arrived in a virgin state and was transparent, suggesting that little oxidation had taken place. Analysis of the chromatograms of the three fog oils yielded no explanation of the differences in coloration.

The generation of oil/water mixtures (OWMs) demonstrated that the fog oil contained mostly water-insoluble components. The low aqueous solubility of the fog oil directly affected our ability to generate toxic stock solutions with high concentrations of fog oil. As a result, only low molecular weight, water soluble compounds were found in solution. Stock solutions generated during Phase II of the project contained fog oil in excess of fog oil's solubility, as indicated by the continued separation of organic and aqueous fractions in the exposure aquaria. Because of the selective solubility of various components of the fog oil, the potential

toxic properties of the OWMs are different than what would be predicted from the overall composition of the bulk fog oil.

Vaporization of fog oil generally resulted in a loss of the more volatile, low molecular weight components of the fog oil. This loss was expected because the fog oil was vaporized at 600°C. Chromatograms of the vaporized fog oil and chromatograms of the bulk fog oil were similar: both had a multitude of peaks and an unresolved envelope. Very small changes in the fog oil as a result of vaporization could cause the observed changes in coloration of the condensed oil; those changes, however, would be obscured in the chromatograms. Few differences between the chromatograms of bulk and vaporized OWMs were noted, indicating again that the changes brought on by vaporization were subtle. Analyses performed during the photolysis experiments indicated that vaporization increased the number of free radicals.

From these investigations, it is apparent that photolysis of fog oils can increase the toxicity of aqueous extracts of the oils through two mechanisms. Production of polar materials that act as emulsifiers can create water-soluble fractions that have much higher concentrations of oil. The higher concentrations occur because surface tension is reduced and fewer suspensions of oil droplets are produced. In this case, the toxic agent consists of fractions of the oil itself. On the other hand, photolysis can result in the production of chemicals (such as aromatic ketones) that have toxic properties apart from those of the original fog oil.

Two types of adverse effects could result from the release of fog oil into aquatic systems: physical effects caused by contact with the oil, and toxicity caused by the chemical constituents of the oil. Toxicity could result from exposure to soluble components of the oil or to hydrophobic oil droplets. The low solubility of fog oil constituents in aqueous solution limits the toxicity of the material to aquatic organisms. The solubility of the oil is determined by the selective extraction of the low molecular weight compounds found in both the bulk and vaporized fog oils. Based on total oil in water, the "solubility" of the oil was estimated at ~30 to 300 µg/L. All stock solutions used during Phase II of the project exceeded this observed level of solubility. It is therefore difficult to interpret the data because the total oil values overestimate the chemical constituents that the aquatic organisms are exposed to over the duration of exposure. The problem is minor, however, because toxicity associated with these oversaturated exposure solutions was observed only when stock solutions exceeded 10 mg/L for the invertebrate species. Thus, the adverse effects are probably due to physical interactions with the fog oil.

4.2.3 Aquatic Toxicity

The tests with fog oil and D. magna provide an estimate of the concentrations of fog oil that could affect a natural population of filter feeding invertebrates. Nominal exposure concentrations as low as 30 µg/L

caused floaters in the Phase II tests with D. magna. In order for these observations of floaters to have any ecological significance, a mechanism for mixing the fog oil in the water column must be present. Mixing energies derived from wind action on lakes and from the natural flow and turbulence of streams are sufficient to create oil-water dispersions similar to those created by the OWMs generated in the laboratory. Thus, effects similar to those demonstrated in the laboratory are possible.

Except for toxicity tests with algae, no discernible differences were evident among the three batches of fog oil. All three batches of fog oil showed some degree of growth inhibition after 96 hr of exposure to the stock solutions (≥ 2.3 mg/L total oil); however, the inhibition was significant in only two of the three tests. The lack of significance in the Batch 3 test may be the result of weaknesses in the experimental design, specifically the use of three replicate flasks per treatment. Because the exposure flasks were routinely shaken every day, it is assumed that the separation of oil from the water column was less than that in other static exposure systems. In tests with algae, it is not possible to separate physical and toxicological effects of fog oil.

Based on the collective results of all the fog oil tests, we conclude that there were no differences in the toxicological properties of the three batches of fog oil. Vaporization of the fog oil in the presence of O_2 caused subtle changes in the chemistry of the fog oil, but these changes did not affect the toxic properties of the fog oil. The slight variations and trends noted in the tests with vaporized fog oil were well within the bounds of experimental error for the number of tests performed.

The potential toxicity of fog oil may be "put in perspective" by comparing the toxicity data of other complex organic liquids to D. magna (Dauble et al. 1983) with the results of our tests (Table 54). This comparison is appropriate because the tests with complex organic liquids were performed at PNL using the same method of preparing OWM preparation. On the basis of both solubility and toxicity, we conclude that the hazard to aquatic life posed by SGF No. 2 fog oil is comparable to or less than Prudhoe Bay crude oil or No. 2 Diesel fuel oil.

The 48-hr LC50 values in Table 62 are based on total carbon measurements of the exposure solutions. For the purposes of this comparison, concentrations of fog oil determined by IR analysis were assumed to be equivalent to total carbon measurements used in the tests reported by Dauble et al. (1983). The materials tested include a number of materials derived from coal liquifaction, including process materials from Solvent Refined Coal (SRC), Integrated Two-Stage Liquifaction (ITSL), and Exxon Donor Solvent (EDS) technologies. These materials contain a high concentration of phenols, hence the relatively high concentration of soluble total carbon in the water soluble fraction (WSF). The WSF is comparable to the OWM. The stock solutions listed in Table 62 represent the soluble components of the stock solutions. A stock solution of fog oil would have a

TABLE 62. COMPARATIVE TOXICITY TO DAPHNIDS OF INTEGRATED TWO-STAGE LIQUIFACTION (ITSL), EXXON DONOR SOLVENT (EDS), AND SEVERAL REFERENCE MATERIALS^a

Material	Concentration of WSF (mg/L) ^c	48-hr LC50 ^b	
		Dilution (%)	TC ^c (mg/L)
SRC-II distillate blend	1068	0.6	6.3
ITSL - LCF feed	7	71.6	4.9
ITSL - liquid product	9	85.0	7.4
ITSL - distillate 650°F	124	7.6	9.5
EDS process solvent once through	103	4.7	5.1
EDS process solvent bottoms recycle	161	3.8	6.2
Shale oil	65	10.4	16.0
No. 2 diesel fuel	9	None at 100% WSF	--
No. 6 bunker feed stock	5	~47.0	~2.4
Prudhoe Bay crude oil	3	None at 100% WSF	--

^aAdapted from Dauble et al. (1983).

^bRelative solubility is indicated by total carbon (TC) in water soluble fractions (WSFs) generated under similar conditions.

^cLC50 and WSF values are given as the mean of reported values.

comparative concentration of 0.2 to 0.3 mg/L as total oil. This level of fog oil is high enough to cause physical impairment to filter feeding organisms but is an order of magnitude lower than the concentrations detrimental to algae and two orders of magnitude lower than the concentrations having a detrimental effect on non-filter feeding invertebrates tested in this project.

Our investigations also revealed that photolysis of fog oil affects toxicity by increasing the solubility of certain constituents. The availability of these constituents to aquatic organisms increases with increasing

aqueous solubility. The absolute concentration of any one of the numerous oxidized constituents in the exposure solutions was very low and would not account for the observed toxicity unless the constituent was highly toxic. We conclude that the observed toxicity was in response to the collective effects of the soluble constituents. No information on the aquatic toxicity of the compounds identified in the 7-day photolyzed fog oil are readily available. No information was available for ketones. The concentrations of photolyzed fog oil capable of eliciting acutely toxic responses in aquatic organisms are still relatively high compared to the toxicity of liquids derived from coal.

4.3 FIELD EXTRAPOLATIONS

This section discusses factors that may have the greatest impact on estimating the potential hazards these materials present to aquatic organisms. Generally, these factors include the amount of smoke generated, the duration of generation, and the volume and surface area of the receiving waters. For phosphorus-based obscurants, the buffering capacity of the receiving water is a major factor controlling toxicity.

4.3.1 Phosphorus-Based Obscurants

A general relationship between total P, buffering capacity and pH can be developed from the different types of waters used in this project, (Table 63). The relationship was developed by selecting the total P values that resulted in a pH value of 6.0 in the various types of water. The data points were taken directly from exposure solutions with a pH of 6.0 or were visually estimated from curves showing the relationship between total P and pH in each dilution water. It should also be noted that this endpoint falls within the lower buffering range of bicarbonate ions. Major decreases in pH will not occur until the buffering capacity of the receiving water has been exceeded. The selection of pH 6.0 was arbitrary and is used in this analysis simply as a point of reference. Acute toxicity, as indicated by 48- and 96-hr LC50 values, occurs at lower pH levels.

The relationship between the deployment of phosphorus-based obscurants, deposition into a body of water, and the resulting toxicity to aquatic organisms is complex. The key variables are the concentration of smoke generated, the deposition velocity, the duration of smoke generation, the size of the body of water that is exposed (surface area, volume and flow rate for streams), and the buffering capacity of the receiving water. The development of a complex model of these parameters is beyond the scope of this project; however, a general relationship based on the following simplified assumptions is possible:

- 1) The column of smoke generated is 20 m high and has a uniform smoke concentration of 300 mg/m³.

TABLE 63. RELATIONSHIP BETWEEN BUFFERING CAPACITY AND TOTAL PHOSPHOROUS AT pH 6.0

Media	Obscurant	Buffering Capacity (CaCO ₃ mg/L)	Total-P (mg/L)	pH
Algae Media	WP-F	16	2.2	6.0
Algae Media	WP-F	16	7.5	6.06
Algae Media	RP-BR	16	2.85	6.08
Reconstituted Soft	WP-F	32.5	12 ^a	6.0
Reconstituted Soft	RP-BR	38	14 ^a	6.0
River	WP-F	65	20.7	6.15
River	RP-BR	65	23.8	6.00
Well	WP-F	115	62 ^a	6.0
Well	WP-F	115	57 ^a	6.0
Well	RP-BR	117	62 ^a	6.0
Reconstituted Hard	WP-F	117	65.2	6.00
Reconstituted Hard	WP-F	129	35.4	5.99
Reconstituted Hard	RP-BR	124	62 ^a	6.0
Reconstituted Hard	RP-BR	112	58 ^a	6.0
Reconstituted Very Hard	WP-F	227	111 ^a	6.0
Reconstituted Very Hard	WP-F	220	104 ^a	6.0

^a Estimated total P at pH 6.0.

- 2) The smoke screen is instantaneously generated and instantaneously settles into a body of water with a settling efficiency of 100%.
- 3) The relationship between total P, pH, and buffering capacity of the receiving water is defined by the following regression equation based on the amount of total P required to lower the pH of a receiving water of X buffering capacity to 6.0:

$$y(\text{mg/L total P}) = 0.51 X (\text{mg/L CaCO}_3 \text{ alkalinity}) - 4.44$$

The coefficient of correlation for this relationship is 0.94.

For example, assume that a 3000-m² pond with a mean depth of 1.5 m has been exposed to a phosphorus smoke screen. The pond has an alkalinity of 100 mg/L CaCO₃ and contains 4.5 x 10⁶ L. The amount of smoke above the pond amounts to 18 kg of smoke distributed in 60,000 m³ of air. Assuming that all the smoke settles into the pond, the resulting total P concentration would be 4 mg/L. According to the regression equation, the estimated amount of phosphorus necessary to lower the pH of the pond to a pH of 6.0 is 51 mg/L. Consequently, more than 10 times the amount of smoke found in the smoke screen would be necessary to lower the pond to a pH of 6.0. The actual amount of smoke deposited in the water, however, would depend on wind speed and its effect on deposition velocity. Other variables would also influence the change in pH of a pond exposed to phosphorus smoke. For example, the conditions under which the smoke was generated could radically affect the speciation of phosphorus combustion products in the smoke. Relative humidity, photolysis, and aging of the smoke before it settles on the water could affect the potential for acidification. Prolonged contact with soil or suspended dust may accelerate the decomposition of the more complex polyphosphates to orthophosphate. The dust or soil particles may adsorb and buffer smoke constituents, thus minimizing the potential for acidification if they were to be washed into a body of water. For these reasons, a scenario of aquatic hazards based on surface runoff into a body of water was not addressed further.

4.3.2 Fog Oil

The primary detrimental effect posed to aquatic organisms by the release of fog oil is physical contact with the oil. As indicated by the low concentrations of oil that cause the "floater" phenomenon with first instar *D. magna*, small amounts of fog oil can cause potential problems. The potential for these types of effects to occur in aquatic habitats depends on a number of variables, such as the characteristics of the receiving water, the amount of fog oil released, and the mode of release (spillage or vapor settling). An exercise analogous to the one presented for phosphorus-based obscurants can be conducted using the first two assumptions; however, because fog oil will float on the surface, initial effects would be restricted to the upper layers of a lake or stream until sufficient time had elapsed for fog oil to disperse into other zones of the lake or stream. A uniform distribution of fog oil in the water column cannot be assumed, nor can a mean concentration of dissolved fog oil in water.

From a toxicological point of view, neither the bulk nor the vaporized fog oil appears to be acutely toxic. The potential for acute detrimental environmental effects would be underestimated without consideration of the effects photolysis has on the solubility and potential toxicity of fog oil.

More information is needed to assess adequately the potential environmental problems associated with photolytic decomposition of fog oils. The products of fog oil photodecomposition have only been investigated in a

preliminary fashion, consistent with the scope of the toxicity testing program. Although aromatic ketones of intermediate molecular weight have been identified, other types of products such as ethers and carboxylic acids are probably formed. In addition, compounds with lower and higher molecular weights were not identified or analyzed in these studies, and are undoubtedly formed by photolysis.

We have no data that relate the amount of solar radiation to the amounts of oxygenated products produced by photolysis. Quantitative methods for determining product formation with time should be developed, taking into account the quantity of fog oil which would be exposed to sunlight during a typical smoke screen exercise. Both mathematical studies and wind tunnel experiments should be conducted to determine the efficiency of the photolysis reactions in an aerosol situation. In addition, models need to be developed that relate quantity of photolyzed oil to environmental perturbations. Further, the potential impact of fog oil photolysis on terrestrial systems, both plant and animal, should be studied.

From the perspective of "acute" effects, the potential hazards to aquatic organisms are relatively low. In contrast, we know very little about the long-term effects of chronic releases of fog oil in freshwater aquatic systems. Many processes such as photolysis, dissolution, sedimentation, biological transformation, concentration of lipid-soluble toxins, and food chain transport ultimately influence the disposition of oils in the environment (Liss-Suter 1978). Most of the research in this area deals with oil spills in the marine environment. Available information on the long-term fate of fog oil, with its distinctive chemical composition, is limited. More research addressing the environmental fate and effects of fog oil in freshwater systems is needed to define and model these processes.

5.0 CONCLUSIONS AND RESEARCH RECOMMENDATIONS

Several major conclusions and recommendations can be drawn from this study.

1. The primary mode of toxicity of the phosphorus-based obscurants results from acidification of the receiving water. Susceptibility of aquatic organisms to Phosphorus-based obscurants was determined by the inherent buffering capacity of the receiving waters.
2. None of the constituents of RP-BR or WP-F smoke were toxic except at high concentrations of total P. The accumulation of tri-meta-phosphate over time suggests that chronic studies with the compound are needed to define the potential hazard it presents to aquatic organisms.
3. SGF No. 2 Fog Oil does not exert any apparent chemical toxicity to aquatic organisms. Physical impairment of aquatic organisms is the greatest adverse effect. The potential effects that photolysis has on the production of water-soluble constituents and their resulting toxicity needs additional research, particularly in the area of actual photolysis of the aerosol and subsequent settling and dissolution in water.

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APPENDIX A
TOXICITY DATA FOR ALGAE TESTS

TABLE A.1. MEAN CELL COUNTS (± 1.0 SD) FOR SELENASTRUM CAPRICORNUTUM
EXPOSED TO BATCH 1 FOG OIL

Treatment (% stock)	Total Oil (mg/L)	Mean Cells Count ($\times 10^3$ /mL)				
		0 hr	24 hr	48 hr	72 hr	96 hr
100	3.23	8.96	22.51	23.50	77.01	348.45
		1.00	3.88	2.11	19.86	118.98
60	2.15	16.76	25.09	19.10	80.74	633.25
		11.35	2.56	7.39	12.56	95.55
40	1.66	8.61	24.32	29.14	99.79	1060.45
		1.73	3.33	3.23	50.65	115.56
20	0.95	7.81	27.42	30.04	91.05	1048.27
		0.67	2.95	9.47	18.80	212.58
10	0.67	10.65	24.45	46.93	148.36	1282.03
		2.83	4.96	7.31	9.06	440.57
5	0.76	8.33	21.51	27.72	183.29	1103.31
		3.23	2.42	2.33	85.21	99.75
Control	0.14	9.69	29.80	75.21	214.49	1004.21
		4.72	1.18	4.97	82.97	838.37

TABLE A.2. MEAN CELL COUNTS (± 1.0 SD) FOR SELENASTRUM CAPRICORNUTUM
EXPOSED TO BATCH 2 FOG OIL

Treatment (% stock)	Total Oil (mg/L)	Mean Cell Count ($\times 10^3$ /mL)				
		0 hr	24 hr	48 hr	72 hr	96 hr
100	2.39	199.82	411.71	692.30	1284.38	2242.66
		16.51	221.38	89.78	682.91	127.89
80	1.44	212.28	241.44	986.76	1569.93	2587.53
		22.25	31.16	113.39	3.21	168.54
40	1.17	210.63	295.13	1099.98	2047.65	3672.06
		15.15	28.61	87.73	229.83	203.84
20	0.65	205.72	302.80	1096.00	2253.73	2757.70
		19.72	7.88	81.92	150.16	1278.41
10	0.45	200.94	365.36	1246.11	2507.90	3859.32
		19.38	20.84	59.12	71.81	295.79
5	0.29	214.16	437.57	741.56	1681.73	2772.08
		28.14	28.95	237.86	551.85	761.98
0	0.20	203.86	372.11	1719.68	2544.48	3207.42
		16.15	28.71	54.83	210.47	163.84

TABLE A.3. MEAN CELL COUNTS (± 1.0 SD) FOR SELENASTRUM CAPRICORNUTUM
EXPOSED TO BATCH 3 FOG OIL

Treatment (% stock)	Total Oil (mg/L)	Means Cell Counts ($\times 10^3$ /mL)				
		0 hr	24 hr	48 hr	72 hr	96 hr
100	3.47	18.28	19.51	64.41	385.17	604.09
		10.97	14.97	41.22	277.46	584.79
60	2.75	13.77	27.01	55.29	364.91	822.25
		9.59	15.04	43.53	258.81	722.98
40	2.56	13.09	16.99	60.61	356.12	1268.01
		10.33	15.17	42.71	262.95	973.66
20	1.07	9.91	19.56	57.76	298.53	1064.74
		7.08	14.46	44.80	318.58	754.23
10	0.61	5.61	17.18	50.65	336.91	1100.70
		6.34	12.60	40.17	248.17	783.01
Control	0.09	6.80	20.68	65.61	397.33	1064.94
		4.99	15.61	47.64	296.37	808.30

TABLE A.4. MEAN CELL COUNTS (± 1.0 SD) FOR SELENASTRUM CAPRICORNUTUM
EXPOSED TO VAPORIZED BATCH 3 FOG OIL

Treatment (% stock)	Total Oil (mg/L)	Mean Cell Count ($\times 10^3$ /mL)				
		0 hr	24 hr	48 hr	72 hr	96 hr
100	2.38	21.42 2.54	40.27 1.89	88.87 4.20	238.66 160.07	828.56 162.32
60	1.48	18.44 1.56	43.55 6.69	98.32 16.79	369.43 176.00	1021.40 500.59
40	1.10	23.01 2.39	47.39 3.54	112.57 10.99	364.43 91.01	948.11 359.07
20	0.76	21.17 0.60	45.68 1.28	111.97 5.67	368.57 167.14	971.52 170.31
10	0.68	24.79 3.06	48.61 6.11	116.27 33.45	461.88 35.75	1349.79 40.20
Control	0.18	26.34 4.73	45.53 2.52	131.42 14.48	429.53 51.45	837.57 103.46

TABLE A.5. MEAN CELL COUNTS (± 1.0 SD) FOR SELENASTRUM CAPRICORNUTUM
EXPOSED TO RP-BR SMOKE-DOSED WATER

Treatment (% stock)	Total Oil (mg/L)	Mean Cell Count ($\times 10^3$ /mL)				
		0 hr	24 hr	48 hr	72 hr	96 hr
100	97.0	13.41 1.07	13.09 0.64	12.71 0.82	6.78 4.08	28.31 23.61
10	9.3	20.90 2.94	15.71 0.93	16.78 1.05	11.71 4.74	17.73 1.96
5	8.6	23.34 10.78	17.23 1.19	15.12 2.09	10.75 3.04	23.01 15.39
7	6.1	20.05 3.86	22.83 4.31	16.69 3.17	5.45 1.19	63.05 69.85
5	4.6	16.16 0.99	44.10 5.23	166.03 11.30	100.95 70.77	3363.16 797.44
1	1.0	17.91 0.83	40.05 8.73	93.54 44.30	38.61 26.15	573.93 801.16
Control	0	17.38 3.38	40.95 7.47	118.50 23.28	103.01 79.48	195.20 122.83
100 ^a	75	25.14 7.90	29.72 2.83	24.76 2.53	19.63 11.36	47.94 6.94

^aNeutralized stock solution with 360 mg/L total alkalinity (RP-7).

TABLE A.6. MEAN CELL COUNTS (± 1.0 SD) IN ALGAL STIMULATION TESTS
WITH RP-BR SMOKE-DOSED WATER

Treatment (% stock)	PO_4^{3-} (mg/L)	pH	Mean Cell Count ($\times 10^3$ /mL)				
			0 hr	24 hr	48 hr	72 hr	96 hr
NA ^a	0.28	7.46	13.28	34.71	97.63	320.94	460.40
			4.29	4.22	24.39	151.84	170.30
NA ^b	0.69	7.50	25.47	35.75	143.97	461.38	832.30
			6.27	2.85	20.54	84.81	209.17
4.98	1.41	4.95	25.40	19.07	18.36	19.37	13.02
			6.87	3.50	3.33	3.36	7.64
3.27	1.13	5.20	24.30	38.67	100.01	257.31	645.36
			3.64	5.58	21.30	81.77	254.75
2.77	0.86	6.08	15.61	20.06	158.31	416.72	1236.78
			3.53	4.60	11.10	77.06	152.55
1.81	0.61	6.48	16.92	34.34	123.09	427.25	1477.70
			0.75	5.58	20.38	90.70	127.30
4.98	1.38	7.20	28.85	48.55	156.90	477.47	1591.26
			13.63	10.95	9.79	41.07	162.85
3.27	0.92	7.15	23.18	40.20	177.79	477.83	1454.92
			2.21	7.29	54.16	207.10	83.28
2.77	0.83	7.24	20.91	46.55	139.03	410.24	1257.10
			4.91	10.49	2.01	27.72	205.65
1.81	0.64	7.25	23.05	43.29	135.44	466.36	1352.52
			1.83	10.94	15.98	13.28	89.76

^aNot applicable, PO_4^{3-} -deficient control.

^bNot applicable, complete media.

TABLE A.7. MEAN CELL COUNTS (± 1.0 SD) FOR SELENASTRUM CAPPICORNUTUM
EXPOSED TO WP-F SMOKE-DOSED WATER

Treatment (% stock)	Total P (mg/L)	Mean Cell Count ($\times 10^3$ /mL)				
		0 hr	24 hr	48 hr	72 hr	96 hr
100	125	11.56	7.44	10.40	8.35	22.71
		3.94	4.58	2.17	2.69	16.09
6	7.6	12.64	11.67	14.19	28.26	46.68
		2.91	6.60	7.96	7.81	35.38
3	3.6	12.79	8.25	25.71	163.19	663.80
		3.82	6.27	17.53	137.44	554.33
1	1.8	11.89	6.78	22.07	105.97	431.53
		1.40	3.15	6.24	114.22	482.24
0.6	0.7	13.44	7.98	21.64	86.90	256.06
		1.55	5.94	14.45	136.40	423.02
Control	DL ^a	13.62	9.82	16.37	46.17	417.41
		2.25	0.43	12.51	59.14	687.94
Neutralized Stock	125	14.24	9.20	16.01	18.74	20.03
		1.15	1.399	1.80	4.39	4.52

^aDetection limits.

TABLE A.8. MEAN CELL COUNTS (± 1.0 SD) IN ALGAL STIMULATION TESTS
WITH WP-F SMOKED-DOSED WATER

Treatment (% stock)	PO ₄ (mg/L)	pH	Means Cell Count ($\times 10^3$ /mL)				
			0 hr	24 hr	48 hr	72 hr	96 hr
NA ^a	0.20	7.5	21.50 2.33	70.42 16.16	113.87 28.10	237.15 54.91	325.88 129.81
NA ^b	--	7.38	20.72 3.18	35.60 12.44	62.85 36.86	330.72 176.56	655.34 277.49
10	3.41	4.8	23.79 2.80	21.36 3.91	13.81 2.12	16.82 7.90	24.04 11.23
7	2.85	5.5	24.63 2.12	22.01 2.46	25.00 2.71	28.65 8.63	48.86 36.07
5.2	2.08	6.0	23.10 2.48	34.00 16.71	69.03 23.74	189.86 58.86	494.47 296.27
2.4	0.89	6.6	27.22 7.86	37.10 16.00	100.03 24.11	724.56 211.06	628.40 291.39
10	3.83	7.35	24.49 3.30	24.30 2.61	103.01 46.86	323.37 47.33	504.27 383.02
7	2.70	7.35	23.44 3.48	50.30 23.64	83.19 23.64	350.42 26.45	197.20 90.23
5.2	2.05	7.35	25.73 3.38	42.72 6.98	121.68 40.88	542.18 132.74	237.74 140.19
2.4	1.20	7.35	24.24 1.79	39.70 12.02	89.40 12.62	420.72 39.38	373.55 150.06

^aNot applicable, PO₄³⁻ deficient control.

^bNot applicable, complete media.

TABLE A.9. RESPONSE (± 1.0 SD) OF SELENASTRUM CAPRICORNUTUM TO RP-BR SMOKE DOSED WATER, CHLOROPHYLL DATA AT 48 AND 96 HR

Treatment (% stock)	Total P (mg/L)	pH	Chlorophyll A (ng/mL)	
			48 hr	96 hr
100	97	2.72 ^a	0.47 \pm 0.02	5.08 \pm 4.83
10	9.3	5.03 ^a	1.56 \pm 0.07	1.17 \pm 0.70
9	8.6	4.95 ^a	1.86 \pm 0.42	5.28 \pm 5.11
7	6.1	5.70 ^a	1.71 \pm 0.27	12.11 \pm 13.84
5	4.6	7.04 ^a	36.55 \pm 1.86	736.30 \pm 42.02
1	1.0	7.76 ^a	13.76 \pm 7.37	50.45 \pm 46.13
Control	<DL	7.8	15.87 \pm 0.55	19.88 \pm 4.98
100 ^c	75	7.9	2.06 \pm 0.18	2.41 \pm 1.20

^apH levels measured at end of test (RP-8).

^bBelow detection limits.

^cNeutralized stock solution with 360 mg/L total alkalinity (RP-7).

TABLE A.10. RESPONSE (± 1.0 SD) OF SELENASTRUM CAPRICORNUTUM TO WP-F SMOKE DOSED WATER, CHLOROPHYLL DATA AT 96 HR

Treatment (% stock)	Total P	pH	Chlorophyll A
			(ng/mL)
100	125	2.55	3.34 \pm 2.12
6	7.6	6.06	8.46 \pm 9.73
3	3.6	6.26	40.06 \pm 33.63
1	1.8	6.77	188.43 \pm 270.57
0.5	0.7	7.02	163.89 \pm 231.04
Control	<DL ^a	7.44	12.81 \pm 13.80
100% Neutralized	125	7.32	0.78 \pm 0.13

^aBelow detection limit.

TABLE A.11. COMPARISON OF ALGAL TOXICITY OF THREE BATCHES OF SGF NO. 2 FOG OIL BASED ON MEAN CHLOROPHYLL ANALYSIS (± 1.0 SD) AT 96 HR

Treatment (% stock)	Batch 1		Batch 2		Batch 3	
	Total Oil (mg/L)	Chlorophyll A (ng/mL) 96 hr	Total Oil (mg/L)	Chlorophyll A (ng/mL) 96 hr	Total Oil (mg/L)	Chlorophyll A (ng/mL) 96 hr
100	3.23	45.52 \pm 12.18	2.39	345.19 \pm 75.29	3.47	223.71 \pm 33.7
60	2.15	37.78 \pm 32.45	1.44	93.10 \pm 79.76	2.75	207.12 \pm 30.92
40	1.66	126.97 \pm 33.83	1.17	212.62 \pm 12.08	2.56	171.44 \pm 11.81
20	0.95	184.54 \pm 59.12	0.65	148.02 \pm 61.79	1.07	216.99 \pm 225.79
10	0.07	278.81 \pm 170.81	0.45	219.10 \pm 46.68	0.61	175.86 \pm 102.70
5	0.76	261.38 \pm 33.00	0.29	121.37 \pm 47.76	-- ^a	--
Control	0.14	119.38 \pm 92.21	0.20	146.25 \pm 61.66	0.09	138.97 \pm 38.39

^aFive percent exposure not conducted for Batch 3 fog oil.

TABLE A.12. RESPONSES (± 1.0 SD) OF SELENASTRUM CAPRICORNUTUM TO VAPORIZED
FOG OIL, CHLOROPHYLL A DATA TAKEN AT 96 HR

Treatment (% stock)	Total Oil (mg/L)	Chlorophyll A (ng/mL)
100	2.38 \pm 0.07	53.91 \pm 17.52
60	1.48 \pm 0.06	58.97 \pm 33.81
40	1.10 \pm 0.09	48.97 \pm 8.15
20	0.76 \pm 0.07	47.78 \pm 23.35
10	0.68 \pm 0.00	64.89 \pm 10.21
0	0.18 \pm 0.02	16.77 \pm 1.61

APPENDIX B

ALGAL MEDIA

TABLE B.1. MEDIA USED FOR TOXICITY TESTING (MILLER ET AL. 1978)

Compound	Concentration (mg/L)	Compound	Concentration (g/L)
H_3BO_3	185.520	$NaNO_3$	25.500
$MnCl_2 \cdot 4H_2O$	415.610	$NaHCO_3$	15.000
$ZnCl_2$	3.271	K_2HPO_4	1.044
$CoCl_2 \cdot 6H_2O$	1.428	$MgSO_4 \cdot 7H_2O$	14.700
$CuCl_2 \cdot 2H_2O$	0.012	$MgCl_2 \cdot 6H_2O$	12.164
$Na_2 \cdot MoO_4 \cdot 2H_2O$	7.260	$CaCl_2 \cdot 2H_2O$	4.410
$FeCl_3 \cdot 6H_2O$	160.000	$Na_2EDTA \cdot 2H_2O^b$	300.000

^aMedia prepared using the following steps:

1. Add 1 mL of each of the 15 stock solutions in the order given to approximately 900 mL of distilled or deionized water and then dilute to 1 L.
2. Adjust pH to 7.5 ± 0.1 with 0.1N NaOH or HCl.

^bOmit if test material is or contains a metal.

TABLE B.2. MEDIA USED TO GROW SELENASTRUM CAPRICORNUTUM TO FEED DAPHNIA
MAGNA (NICHOLS AND BOLD 1965)

A. Macronutrients - prepare six separate stocks, add 10 mL each to a final volume of 1.0 L.

1) NaNO_3	10 g/400 mL
2) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1 g/400 mL
3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3 g/400 mL
4) K_2HPO_4	3 g/400 mL
5) KH_2PO_4	7 g/400 mL
6) NaCl	1 g/400 mL

B. Minor constituents - prepare three separate stocks, add 1 mL each to a 1.0 L of media.

1) EDTA	50 g/L
KOH	31 g/L
2) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	4.98 g/L
H_2SO_4	1.0 ml/L
3) H_3BO_3	11.42 g/L

C. Micronutrients - prepare one stock solution containing the following compounds, add 1.0 mL to 1.0 L media.

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.82 g/L
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.44 g/L
MoO_3	0.71 g/L
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	1.57 g/L
$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	0.49 g/L

TABLE B.2. (continued)

D. Vitamins - prepare one stock solution composed of the following vitamins, add 1.0 mL to 1.0 L media (Goulden et al. 1982).

Biotin	5 mg/L
Thiamine	100 mg/L
Pyridoxine	100 mg/L
Pyridoxamine	3 mg/L
Calcium pantothenate	250 mg/L
B ₁₂ (as 0.1% mannitol)	100 mg/L
Nicotinic Acid	50 mg/L
Nicotinamide	50 mg/L
Folic Acid	20 mg/L
Riboflavin	30 mg/L
Inositol	90 mg/L

APPENDIX C

TOXICITY TEST DATA PHOSPHORUS BASED OBSCURANTS

This appendix contains summary data from toxicity tests conducted on this project. This information includes toxicant concentration, pH, daily mortality observations and, for fish tests, un-ionized NH_3 values. Dissolved oxygen values did not fall below 5.0 mg/L except in the first test with *I. punctatus* (Table C.13). Aeration was provided in that test after 72 hr and for the duration of the second RP-BR test (Table C.12).

TABLE C.1. RP-BR NEUTRALIZATION TESTS WITH DAPHNIA MAGNA IN WELL WATER, BURN RP-5 (TOTAL P = 93 mg/L IN STOCK SOLUTION)

Treatment (% Stock)	pH ^a	Mortality (%)			
		24 hr	48 hr	72 hr	96 hr
Neutralized					
100	7.43	0	0	4.2	75
70	7.76	0	0	0	12.5
50	7.85	0	0	12.5	66.7
40	8.04	0	0	20.8	62.5
30	8.12	0	0	4.2	20.8
10	8.24	0	0	0	0
Non-Neutralized					
100	3.34	100	100	100	100
70	5.47	33.3	91.7	100	100
50	6.82	0	0	4.2	83.3
40	7.43	0	0	16.7	79.2
30	7.73	0	0	0	29.2
10	8.06	0	0	0	0
Control	8.27	0	0	0	0

^a pH taken at 24 hr.

TABLE C.2. RP-BR NEUTRALIZATION TESTS WITH DAPHNIA MAGNA IN RECONSTITUTED
SOFT WATER, BURN RP-9 (TOTAL P = 70 mg/L IN STOCK SOLUTIONS)

Treatment (% Stock)	Initial pH	Mortality (%)			
		24 hr	48 hr	72 hr	96 hr
Neutralized					
100	7.25	45	100	100	100
20	7.19	0	0	0	0
10	7.25	0	0	0	0
5	7.32	0	0	5	5
2	7.37	0	0	0	0
0	7.32	0	0	0	0
Non-Neutralized					
100	2.92	100	100	100	100
20	5.73	0	0	5	5
10	6.44	0	0	0	0
5	6.80	0	0	0	0
2	7.04	0	0	0	0
0	7.25	0	0	0	0

TABLE C.3. RP-BR NEUTRALIZATION TEST WITH DAPHNIA MAGNA IN RECONSTITUTED HARD WATER, TEST 1, BURN RP-15 (TOTAL P = 65.4 mg/L IN STOCK SOLUTION)

Treatment (% stock)	Initial pH	Mortality (%)			
		24 hr	48 hr	72 hr	96 hr
Neutralized					
100	8.14	0	0	10	20
80	8.05	0	0	5	5
60	8.19	0	0	0	0
40	8.24	0	5	5	15
20	8.28	0	0	0	10
0	8.33	0	0	0	25
Non-Neutralized					
100	5.50	25	55	55	55
80	6.47	0	5	20	25
60	6.97	0	0	5	10
40	7.28	0	0	5	5
20	7.70	0	0	0	10
0	8.26	0	0	5	5

TABLE C.4. RP-6R NEUTRALIZATION TESTS WITH DAPHNIA MAGNA IN RECONSTITUTED HARD WATER, TEST 2, BURN RP-16 (TOTAL P = 69.1 mg/L IN STOCK SOLUTION)

Treatment (% stock)	Initial pH	Mortality (%)			
		24 hr	48 hr	72 hr	96 hr
Neutralized					
100	7.96	0	0	5	15
70	8.01	0	0	0	0
50	8.08	0	0	5	5
30	8.15	0	5	5	5
10	8.17	0	0	5	5
Non-Neutralized					
100	4.70	95	100	100	100
70	6.29	0	0	0	5
50	6.76	0	0	0	0
30	7.15	0	0	0	0
10	7.70	0	0	0	0
0	8.20	0	0	0	0

TABLE C.5. RP-BR NEUTRALIZATION TESTS WITH DAPHNIA MAGNA IN RECONSTITUTED VERY HARD WATER, BURN RP-5 (TOTAL P = 96.3 mg/L IN STOCK SOLUTION)

Treatment (% Stock)	pH at 24 hr	Mortality (%)			
		24 hr	48 hr	72 hr	96 hr
Neutralized					
100	7.80	0	0	12.5	25
70	8.10	0	0	0	16.7
50	8.22	0	0	8.3	25
40	8.29	0	0	4.2	25
30	8.40	0	0	4.2	16.7
10	8.50	0	0	0	4.2
Non-Neutralized					
100	7.13	0	0	37.5	91.7
50	8.11	0	0	0	0
40	8.22	0	0	4.2	8.3
30	8.30	0	0	0	0
10	8.47	0	0	0	0
Control ^a	8.51	0	0	5.5	11.1

^aBroke one control beaker, n = 18.

TABLE C.6. CUMULATIVE MORTALITY OF DAPHNIA MAGNA TO RP-BR SMOKE-DOSED RIVER WATER, BURN RP-6

Treatment (% Stock) ^a	Initial pH	Total P (mg/L)	Mortality (%) ^a	
			24 hr	48 hr
100	4.45	34.4	100	100
90	5.23	30.7	60	80
80	5.70	28.2	0	15
70	6.05	23.8	0	0
20	6.82	10.5	0	0
Control	7.73	<1 ^(b)	0	0

^aFive instars per beaker, four beakers.

^bBackground levels.

TABLE C.7. CUMULATIVE MORTALITY OF HYALLELA AZTECA TO RP-BR SMOKE-DOSED RIVER WATER, BURN RP-12

Treatment (% Stock)	Initial pH	Total P (mg/L)	Mortality (%) ^a	
			24 hr	48 hr
91	3.26	60.6	100	100
82	3.69	49.0	100	100
62	3.84	42.1	100	100
59	4.30	36.8	10	80
40	6.98	24.0	0	0
Control	8.04	0.16	0	0

^a Twenty organisms per treatment.

TABLE C.8. ACUTE MORTALITY OF PARATANYTARSUS DISSIMILUS TO RP-BR SMOKE-DOSED WATER, BURN RP-12

Treatment (% Stock)	Initial pH	Total P (mg/L)	48-hr	
			Mortality (%)	Emergents ^a
91	3.26	60.6	70	0
82	3.69	49.0	20	1
62	3.84	42.1	5	3
59	4.37	36.8	5	3
40	6.98	24.0	0	1
Control	8.04	0.16	0	1

^a Assumed to be alive.

TABLE C.9. CUMULATIVE MORTALITY OF HEXAGENIA EXPOSED TO RP-BR SMOKE-DOSED RIVER WATER, BURN RP-13

Treatment (% stock)	Initial pH	Total P (mg/L)	Mortality (%)	
			24 hr	48 hr
50	3.0	73.1	45	100
35	3.43	50.7	15	100
34	3.71 - 3.72	45.5	10	100
33	4.35 - 4.42	39.1	5	10
10	6.63	13.6	0	0
Control	7.92	0.20	0	0

TABLE C.10. CUMULATIVE MORTALITY OF PIMEPHALES PROMELAS EXPOSED TO RP-BR SMOKE-DOSED RIVER WATER (10 FISH/AQUARIUM), BURN RP-10

Treatment (% stock) ^a	Initial pH	Total P (mg/L)	Mortality (%)				96-hr NH ₃ (µg/L)
			24 hr	48 hr	72 hr	96 hr	
Control	8.19	0.25	0	0	0	0	4.2
Control	8.46	0.37	0	0	0	0	4.3
10.0	5.80	24.9	0	0	0	0	0.5
10.0	5.76	22.9	0	0	0	0	0.4
13.6	4.99	31.8	0	10	100	100	ND ^a
13.6	5.41	33.0	0	0	80	80	0.3
14.3	4.26	40.4	100	100	100	100	ND
14.3	4.65	36.8	10	40	100	100	ND
15.0	4.21	41.0	100	100	100	100	ND
15.0	4.32	39.9	100	100	100	100	ND
16.6	3.87	45.7	100	100	100	100	ND
16.6	3.86	45.3	100	100	100	100	ND

^aNot determined.

TABLE C.11. CUMULATIVE MORTALITY OF SALMO GAIRDNERI EXPOSED TO RP-BK
SMOKE-DOSED RIVER WATER, BURN RP-16

Treatment (% stock) ^a	Initial pH	Total P (mg/L)	24 hr	48 hr	72 hr	96 hr	96-hr NH ₃ (µg/L)
25	3.43, 3.65	50.1, 51.0	100	100	100	100	ND ^a
20	4.31, 4.31	40.7, 40.9	0	25	75	85	0.1-0.2
18	4.61, 4.86	35.7, 40.8	0	0	0	5	0.1-0.2
15	5.45, 5.52	29.6	0	0	0	0	0.1-0.5
10	5.96, 6.06	22.1, 22.2	0	0	0	0	0.7-1.2
Control	7.68, 7.75	0.13	0	0	0	0	2.5-4.5

^aNot determined.

TABLE C.12. CUMULATIVE MORTALITY OF ICTALURUS PUNCTATUS EXPOSED TO RP-BR
SMOKE-DOSED WATER, BURN RP-11 (TEST REPEATED)

Treatment (% stock)	Initial ^a pH	Total P (mg/L)	Mortality (%) ^b				96-hr NH ₃ (µg/L)
			24 hr	48 hr	72 hr	96 hr	
25.2	3.42, 3.46	49.9	43.8	100	100	100	<0.1-0.5
21.1	4.08, 4.13	41.7	0	0	0	0	<0.1
18.9	4.68, 4.69	37.5	6.3	6.3	6.3	6.3	<0.1
14.3	5.52, 5.68	28.3	0	0	0	0	9.0-1.0
Control	7.93, 8.00	0.16	0	0	0	0	18.2-23.2

^aValues for duplicate aquaria.

^bSixteen fish per treatment.

TABLE C.13. CUMULATIVE MORTALITY OF LEPOMIS MACROCHIRUS EXPOSED TO
RP-BR SMOKE-DOSED WATER, BURN RP-12

Treatment (% stock)	Initial pH	Total P (mg/L)	Mortality (%)				96-hr Combined	96-hr NH ₃ (µg/L)
			24-hr	48-hr	72-hr	96-hr		
Control	7.8	0.3	0	0	0	0		2.42
Control	7.83	0.14	0	0	20	20	10	2.42
5	5.60	26.6	0	0	0	0	0	0.05
5	5.54	27.1	0	0	0	0	0	0.03
7	4.19	37.9	0	10	10	10	5	0.04
7	4.20	37.6	0	0	0	0		0.03 ^a
8	3.71	31.6	100	100	100	100	100	ND ^a
8	3.68	43.1	80	100	100	100		ND
9	3.49	48.5	100	100	100	100	100	ND
9	3.44	49.3	100	100	100	100		ND
11	3.23	59.1	100	100	100	100	100	ND
11	3.22	58.9	100	100	100	100		ND

^aNot determined.

TABLE C.14. NEUTRALIZATION TEST WITH DAPHNIA MAGNA IN WELL WATER, BURN
WP-4 (TOTAL P = 66.4 mg/L IN STOCK SOLUTION)

Treatment (% stock)	Initial pH	Mortality (%)			
		24 hr	48 hr	72 hr	96 hr
Neutralized					
100	7.78	0	4.2	12.5	16.7
70	7.8	0	0	4.2	8.3
60	7.91	4.2	4.2	4.2	12.5
50	7.94	0	0	0	0
30	7.95	4.2	12.5	12.5	29.2
10	7.99	0	0	0	0
Non-Neutralized					
100	5.38	54.2	100	100	100
70	6.67	0	0	4.2	25
60	7.01	0	0	0	12.5
50	7.25	0	0	0	0
30	7.48	0	0	0	0
10 ^a					
Control	7.98	0	4.2	16.7	41.7

^a10% dilution not tested for non-neutralized treatment.

TABLE C.15. WP-F NEUTRALIZATION TEST WITH DAPHNIA MAGNA IN RECONSTITUTED
SOFT WATER, BURN WP-4 (TOTAL P = 66.4 mg/L IN STOCK SOLUTION)

Treatment (% stock)	Mortality (%)				
	pH	24 hr	48 hr	72 hr	96 hr
Neutralized					
100	7.69	38	88	100	100
70	7.48	0	0	8	25
50	7.47	0	4	4	4
40	7.48	0	0	0	4
30	7.50	0	0	0	4
10	6.54	0	0	0	0
Non-Neutralized					
100	2.90	100	100	100	100
70	3.10	100	100	100	100
50	3.38	100	100	100	100
40	3.71	100	100	100	100
30	4.71	100	100	100	100
10	6.63	0	0	0	0
1.0	7.62	0	0	0	0
Control	7.98	0	0	0	3

TABLE C.16. WP-F NEUTRALIZATION TESTS WITH *DAPHNIA MAGNA* IN RECONSTITUTED HARD WATER, BURN WP-10 (TOTAL P = 88.6 mg/L IN STOCK SOLUTION)

Treatment (% Stock)	pH	Mortality (%)			
		24 hr	48 hr	72 hr	96 hr
Neutralized					
100	7.97	20	40	45	50
80	7.98	0	5	5	10
60	8.00	5	10	10	25
40	8.06	0	5	10	25
20	8.04	0	0	0	15
0	8.19	0	0	0	5
Non-Neutralized					
100	3.08	100	100	100	100
80	3.31	100	100	100	100
60	3.91	100	100	100	100
40	5.99	0	10	15	20
20	6.78	0	5	10	10
0	8.14	0	0	10	15

TABLE C.17. WP-F NEUTRALIZATION TEST WITH DAPHNIA MAGNA IN RECONSTITUTED VERY HARD WATER, BURN WP-7 (TOTAL P = 125 mg/L IN STOCK SOLUTION)

Treatment (% Stock)	Initial pH	Mortality (%)			
		24 hr	48 hr	72 hr	96 hr
Neutralized					
100	8.08	0	30	65	80
70	8.31	5	10	25	40
50	8.47	5	5	40	50
30	8.58	0	0	5	25
10	8.64	5	15	25	40
Non-Neutralized					
100		90	100	--	--
70	4.99	0	0	30	55
50	6.55	0	0	5	20
30	7.04	0	5	5	5
10	7.52	0	0	5	10
Control	8.66	0	0	0	0

TABLE C.18. CUMULATIVE MORTALITY OF DAPHNIA MAGNA TO WP-F SMOKE-DOSED RIVER WATER, BURN WP-16

Treatment (% stock)	Initial pH	Total P (mg/L)	Mortality (%)	
			24 hr	48 hr
25	4.48	34.4	100	100
23.5	5.22	30.7	55	80
20.9	5.48	28.2	10	45
18.75	5.82	23.8	0	35
15	6.15	10.5	0	30
Control	8.6	(bkg)	0	0

TABLE C.19. MORTALITY OF HYALLELA AZTECA EXPOSED TO WP-F SMOKE-DOSED RIVER WATER, BURN WP-14

Treatment (% stock)	Initial pH	Total P (mg/L)	Mortality (%)	
			24 hr	48 hr
10.3	3.5	44.4	100	100
8.1	4.31	34.6	5	55
7.9	4.51	33.8	5	55
7.2	4.99	30.9	0	5
6.0	5.5	26.2	0	5
Control	7.66	(Bkg)	0	0

TABLE C.20. MORTALITY AND CASE BUILDING DATA FOR PARATANYTARSUS DISSIMILUS
EXPOSED TO WP-F SMOKE-DOSED RIVER WATER, BURN WP-14

Treatment (% stock)	Initial pH	Total P	Mortality (%) 48 hr	Case Building (total number of tubes)
17.8	2.91	76.4	100	0
12.7	3.21	54.9	95	3
10.3	3.50	44.4	88	10
8.7	3.99	37.2	0	26
6.1	5.53	26.2	0	20
0	7.66	(Bkg)	0	23

TABLE C.21. CUMULATIVE MORTALITY OF HEXAGENIA EXPOSED TO WP-F SMOKE-DOSED
WATER, BURN WP-15

Treatment (% stock)	Initial pH ^(a)	Total P (mg/L)	Mortality (%)	
			24 hr	48 hr
12	3.50, 3.59	49.6	20	100
11	3.80, 3.82	46.6	10	90
10	4.14, 4.33	41.7	5	5
9	4.79, 4.95	37.5	0	5
7	6.02, 6.16	29.2	0	0
Control	7.76, 7.93	0.83	0	0

TABLE C.22. CUMULATIVE MORTALITY OF PIMEPHALES PROMELAS EXPOSED TO WP-F
SMOKE-DOSED RIVER WATER, BURN WP-14

Treatment (% stock)	Initial pH ^a	Total P (mg/L)	Mortality (%)				NH ₃ (µg/L)
			24 hr	48 hr	72 hr	96 hr	
10	3.69, 3.87	44.5	100	100	100	100	ND ^b
9	4.02, 4.04	40.8	100	100	100	100	ND
8	4.65, 4.74	35.1	0	0	0	15 ^c	0.06
6	5.61, 5.85	27.3	0	0	0	0	0.07
5	6.02, 6.03	22.7	0	0	0	0	0.10
Control	7.93, 7.95	(Bkg)	0	0	0	0	1.21

^aValues for duplicate aquaria.

^bNot determined.

^cThree fish showed loss of equilibrium.

TABLE C.23. CUMULATIVE MORTALITY OF SALMO GAIRDNERI EXPOSED TO WP-F
SMOKE-DOSED RIVER WATER, BURN WP-15

Treatment (% Stock)	Initial pH ^a	Total P ^a (mg/L)	Mortality (%)				96-hr NH ₃ (µg/L)
			24 hr	48 hr	72 hr	96 hr	
12%	3.57 - 3.59	49.1, 49.8	100	100	100	100	ND ^(b)
10%	4.13 - 4.19	41.5, 41.5	100	100	100	100	ND
9%	4.63 - 4.77	36.4, 37.2	50	65	100	100	<0.1-0.1
8%	5.23 - 5.26	32.6, 32.7	25	25	25	30	<0.1
7%	5.47 - 5.51	28.3, 28.4	0	0	0	0	0.2-0.3
Control	7.67 - 7.72	C.2	0	0	0	0	8.5-9.7

^aValues for duplicate aquaria.

^bNot determined.

TABLE C.24. MORTALITY OF ICTALURUS PUNCTATUS TO WP-F SMOKED-DOSED RIVER WATER, BURN WP-13

Treatment (% stock)	pH		Total P (mg/L)	Mortality (%)				96-hr NH ₃ (µg/L)
	Initial	96 hr		24 hr	48 hr	72 hr	96 hr	
10	3.31	3.34	59.7	100	100	100	100	ND ^a
9	3.50	3.52	53.3	100	100	100	100	ND
8	3.78	3.99	44.5	5	55	100	100	<0.1
7	4.28	5.08	40.3	5	10	15	15	<0.1
5	5.55	6.74	27.3	0	0	0	0	1.2 - 0.21
Control	7.77	8.02	0.1	0	0	0	0	23.0 - 24.0

^aNot determined.

TABLE C.25. CUMULATIVE MORTALITY OF LEPOMIS MACROCHIRUS EXPOSED TO WP-F SMOKE-DOSED RIVER WATER, BURN WP-16

Treatment (% stock)	Initial ^a pH	Total P ^a (mg/L)	Mortality (%)				96-hr NH ₃ (µg/L)
			24 hr	48 hr	72 hr	96 hr	
8.0	3.66, 3.77	45.9, 47.3	95	95	100	100	<0.10
7.5	3.82, 3.92	44.5, 45.0	30	45	45	50	<0.10
7.0	4.18, 4.66	39.5, 39.9	0	0	0	0	<0.10
6.5	4.79, 4.68	38.0, 39.0	0	0	0	0	<0.10
6.0	5.31, 5.38	34.9, 35.9	0	0	0	0	<0.10
Control	7.91, 7.93	<0.1	0	0	0	0	3.96

^aValues for duplicate aquaria.

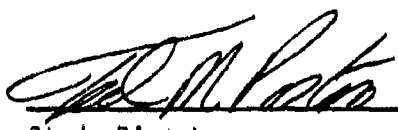
APPENDIX D

PHASE II GLP REQUIREMENTS

Phase II of this study was performed under Good Laboratory Practices as defined by 40 CFR 792.

This appendix contains the Quality Assurance Unit's statement for Phase II studies, a list of personnel assigned to Phase II studies, the study dates for Phase II GLP toxicity tests, and the number of organisms used in Phase II GLP tests.

All raw data and the final report are stored in the LSL-II Building, Rooms 1433 at Battelle, Pacific Northwest Laboratories. Duration of data storage will be in compliance with 40 CFR 792.195 or until the U.S. Army requests transfer of the data, whichever occurs first.


Study Director

March 31, 1986
Date

STUDY PERSONNEL

Study Director	T. M. Poston
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	B. L. Thomas
Phosphorus Chemistry	K. M. McFadden
Aerosol Generation	M. L. Clark
	B. W. Killand
Statistics	L. A. Prohammer
Test Organism Culture	C. S. Abernethy
Toxicity Testing	T. M. Poston
	E. W. Lusty
	M. T. McLane
	T. P. LeMieux

DEVIATIONS IN TEST TEMPERATURE

Exposure temperatures deviated by more than 1°C in the following six tests. Exposure temperatures in the remaining tests did not exceed the range specified in Section 2.

1. Neutralization test with D. magna and RP-BR (Phase I). Initial temperatures in this test were 20.3 to 22.0° C. Temperatures equilibrated at 19.8 to 21.2° C at 24 hr and 18.5 to 20.2° over the remaining 96 hr of the test. The desired test temperature was 20.0 ± 1.0° C.

2. Acute test with H. azteca and RP-BR. Temperatures at 24 hr ranged from 18.4 to 19.1° C and at 48 hr, ranged from 17.9 to 18.2° C. The desired test temperature was 17.0 ± 1.0° C.
3. Acute test with P. dissimulus and RP-BR. Temperatures in two beakers at 48 hr were 21.2 and 21.4° C. All other monitored temperatures ranged from 20.4 to 21.0° C. The desired test temperature was 20.0° ± 1.00 C.
4. Acute test with bluegill sunfish and RP-BR. Temperatures at the start of the test and at 24 hr ranged from 18.2 to 20.0° (control aquaria only), all other aquaria were 19.0° C or less. Exposure temperatures fell within the desired range of 17.0 ± 1.0° C for the remainder of the test.
5. Acute test with rainbow trout and RP-BR. There was one instance where the exposure temperature fell outside of the range of the desired temperature (12.0 ± 1.0° C). At the start of the test, one aquarium registered 10.3° C.
6. Acute test with rainbow trout and WP-F. Exposure temperatures ranged from 10.5 to 12.0° C at the start of the test and 10.5 to 11.0° C at 72 and 96 hr. The desired test temperature was 12.0 ± 1.0° C. Temperatures at 48 hr ranged from 11.0 to 11.4° C.

PHASE II STUDY DATES

	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Number of Organisms</u>
Red Phosphorus-Butyl Rubber			
<u>S. capricornutum</u> - toxicity	09-13-84	09-17-84	NA ^a
<u>S. capricornutum</u> - stimulation	12-10-84	12-14-84	NA
<u>D. magna</u>	08-28-84	08-30-84	120
<u>P. dissimulus</u>	11-14-84	11-16-84	120
<u>H. azteca</u>	11-14-84	11-16-84	120
<u>Hexagenia</u>	12-05-84	12-07-84	120
<u>S. gairdneri</u>	01-21-85	01-25-85	120
<u>P. promelas</u>	11-06-84	11-10-84	120
<u>I. punctatus</u> (Test 1)	11-06-84	11-10-84	120
<u>I. punctatus</u> (Test 2)	12-10-84	12-14-84	100
<u>L. macrochirus</u>	11-14-84	11-18-84	120
White Phosphorus-Felt			
<u>S. capricornutum</u> - toxicity	09-21-84	09-25-84	NA
<u>S. capricornutum</u> - stimulation	11-30-84	12-04-84	NA
<u>D. magna</u>	01-31-85	02-02-85	120

PHASE II STUDY DATES (continued)

	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Number of Organisms</u>
<u>P. dissimilus</u>	11-29-84	12-01-84	120
<u>H. azteca</u>	11-29-84	12-01-84	120
<u>Hexagenia</u>	12-17-84	12-19-84	120
<u>S. gairdneri</u>	12-17-84	12-21-84	120
<u>P. promelas</u>	11-29-84	12-03-84	120
<u>I. punctatus</u>	10-30-84	11-03-84	120
<u>L. macrochirus</u>	01-28-84	02-01-85	120
SGF No. 2 Fog Oil			
Batch 1			
<u>S. capricornutum</u> - toxicity	08-21-85	08-25-85	NA
<u>Hexagenia</u>	11-26-84	11-28-84	60
<u>P. promelas</u>	10-15-84	10-19-84	80
Batch 2			
<u>S. capricornutum</u> - toxicity	08-16-84	08-20-85	NA
<u>Hexagenia</u>	11-19-84	11-21-84	60
<u>P. promelas</u>	10-24-85	10-28-85	40
Batch 3			
<u>S. capricornutum</u> - toxicity	09-17-84	09-21-84	NA
<u>D. magna</u>	02-06-85	02-08-85	160
<u>P. dissimilus</u>	02-06-85	02-08-85	120
<u>H. azteca</u>	01-24-85	01-26-85	48
<u>Hexagenia</u>	11-19-84	11-21-84	60
<u>P. promelas</u>	10-24-84	10-28-85	40
Vaporized Batch 3			
<u>S. capricornutum</u>	12-18-84	12-22-84	NA
<u>D. magna</u>	01-31-85	02-02-85	160
<u>P. dissimilus</u>	01-31-85	02-02-85	120
<u>H. azteca</u>	01-15-85	01-17-85	120
<u>Hexagenia</u>	01-15-85	01-17-85	60
<u>P. promelas</u>	01-02-85	01-06-85	60

^aNot applicable

ACUTE TOXICITY OF SMOKE SCREEN MATERIALS TO AQUATIC ORGANISMS - PHASE II

Quality Assurance Statement

Listed below are phases and/or procedures included in the Phase II Toxicity Tests which were reviewed by Quality Assurance, dates reviews were performed and dates findings reported to management. (Findings were reported to the study director or his designee at the time of the review.)

<u>Test Procedures Reviewed</u>	<u>Review Dates</u>	<u>Date Findings Submitted in Writing to Study Director & Management</u>
Test: <u>S. capricornutum</u> with Batch 2 Fog Oil		
Dose Preparation	08/16/84	-
Dosing	08/16/84	-
Test: <u>S. capricornutum</u> with Batch 3 Fog Oil		
Dose Preparation	09/17/84	09/17/84
Dosing	09/17/84	09/17/84
Test: <u>S. capricornutum</u> with WP-F		
Dose Preparation	09/21/84	-
Sample Collection	09/27/84	09/27/84
Chlorophyll Analysis	10/09/84	10/09/84
Test: <u>P. promelas</u> with Batch 3 Fog Oil		
Dose Analysis	10/29/84	-
Tests: <u>L. macrochirus</u> with RP-BR; <u>P. dissimilis</u> with RP-BR		
Dosing	11/14/84	11/14/84
Test: <u>Hexagenia</u> with RP-BR		
Dosing	12/05/84	-
Evaluation of Toxicity	12/06/84	-
Test: <u>D. magna</u> with WP-F		
Dose Preparation	01/28/85	01/28/85
Tests: <u>P. Dissimilis</u> with Batch 3 Fog Oil; <u>D. Magnia</u> with Batch 3 Fog Oil		
Dose Preparation	02/06/85	-
Dosing	02/06/85	-
Evaluation of Toxicity	02/08/85	-

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ACUTE TOXICITY OF SMOKE SCREEN MATERIALS TO AQUATIC ORGANISMS - PHASE II

Quality Assurance Statement (continued)

<u>Audit</u>	<u>Review Dates</u>	<u>Date Findings Submitted in Writing to Study Director & Management</u>
Data	09/04/84	09/12/84
Data	10/09/84	10/09/84
Data	12/17-19/84	01/07/85
Data	01/25/85	01/25/85
Data	01/09&2/13/86	03/22/86
Final Report	2/14,17&18/86	03/22/86

S. M. Grew
Quality Assurance Specialist

3-27-84
Date

R. L. Suman
Quality Assurance Specialist

3/27/86
Date

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